Environmental Monitoring: Utilizing Efficient Extraction, Separation, and Detection Methods for Application in Routine and Specialized Analyses

by

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Abstract

Environmental analysis is a field that has made significant progress in academic research over the last number of years. Whether it be sampling techniques, sample introduction, separations, or analytical instrumentation, there seems to be no shortage of ideas and innovation to improve the way that environmental analyses could be done on almost any sample type that could be thought of. Interestingly, the field of routine environmental analysis in industry is lagging far behind what we are capable of. Many methods are not only outdated, but they also do not provide the same kind of information that should be capable of them. This is despite breakthroughs that used to be thought of as belonging to "labs of the future" already existing in literature and academia. The question needs to be asked: How can analysis methods be designed so that they are immediately available to the routine environmental analysis industry?

The answer often lies in the transferability of the techniques and methods being used. Many techniques are used for environmental analyses, but this thesis will focus on adaptations of gas chromatography (GC) advancements, especially for multidimensional separations such as comprehensive two-dimensional gas chromatography (GC×GC). Time-of-flight mass spectrometry (TOFMS) is paired with GC×GC for additional identification purposes, when also paired with flame ionization detection (FID) for the most effective quantification of organic compounds. GC×GC-TOFMS is perfectly suited for the effective identification of compounds when using many types of detectors, making it ideal for non-targeted and hybrid target/non-target analysis. This thesis will also focus on a variety of extraction and sample introduction methods for analysis by GC×GC. Being able to analyze many types of samples will help make new routine analyses viable, and being able to adapt multiple methods of sample introduction to a similar method of analysis will help to achieve this viability.

The work in this thesis is designed to help streamline the process of bringing academic advancements to the routine analysis lab in a way that will be fast, economical, and easy to use. The methods designed are meant to modernize routine analysis of environmental samples. One way this is done is through the use of updated extraction methods for aqueous, solid, and headspace matrices. Another way is by introducing multidimensional chromatography and new data analysis tools for more comprehensive analyses.

Preface

A version of **Chapter 2** has been published as Johnson, T.J.; Armstrong, M.D.S.; de la Mata, A.P.; Harynuk, J.J. J Chromatog Open. 2022, 2, 100070. I was responsible for conceptualization and design of the extraction method, all of the experimental work, the formal analysis and data interpretation, as well as the initial manuscript composition and final edits. M.D.S. Armstrong was responsible for programming and conceptualization of analysis methods and algorithms, as well as first edits of manuscripts. A.P. de la Mata was responsible for assistance with time management, supervision and manuscript edits. J.J. Harynuk was responsible for acquisition of funding, conceptualization, supervision and manuscript ideation and edits.

Portions of **Chapter 6**, Section 3 have been published as Dias, R.P.; Johnson, T.A.; Ferrão, L.F.V.; Munoz, P.R.; de la Mata, A.P.; Harynuk, J.J. J of Chrom Open. 2022, 100075. I was responsible for method development, composition of reports that were used in the manuscript, investigation and edits of the manuscript. R.P. Dias also took part in method development and was the main designer of the conceptualization of the project. He was also responsible for data curation, investigation and composition of the manuscript. L.F.V. Ferrão took part in conceptualization, reviewing and editing of manuscripts, supervision and project administration. R.P. Munoz was responsible for funding acquisition, conceptualization, supervision, project administration and provision of resources. A.P. de la Mata took part in conceptualization, reviewing and editing of manuscripts, supervision, and project administration. J.J. Harynuk was responsible for funding acquisition, conceptualization, review and editing of the manuscript, supervision, project administration and provision of resources within the lab.

Dedication

To Grandma

My inspiration to love scholarship and learning.

My chemistry matriarch.

You knew that I could do anything.

Acknowledgements

I would first like to thank my supervisor, Dr. James Harynuk who welcomed me into his research group over five years ago now. I am extremely grateful to have found a group and set of projects that fit so well with the research that have done in the past and the research that I see myself doing in the future. I am especially grateful for his guidance and his mentality to put his team and his students first. The COVID-19 pandemic hit right in the middle of my degree, but I was still able to attend the CSC in Edmonton (before the pandemic and its name change) and CCCE in Calgary (after the pandemic and its name change), and was game for my crazy idea of presenting two of my projects at the XXIV International Symposium on Advances in Extraction Technologies in Iquique, Chile. It was also a great idea to spend a weekend retreat with the group in Canmore, Alberta this January, even though I am sad this is only planned to become a regular occurrence after I leave the group.

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Thank you to all of my friends here in Edmonton or back in Kamloops, Victoria, Vancouver, Ireland, India or wherever else you may be. I have felt your support and companionship, even if we can't be in touch

all of the time. A special mention goes to the members of UNG, who have provided me with many opportunities to relieve stress, talk philosophy/business/politics, and introduced me to the fascinating world of League of Legends and e-sports. Thank you to Yoojin, who has been a shining light, and the best Support-carry, bird mom and overall companion that a guy could ask for.

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List of Abbreviations

GC	-	Gas chromatograph/chromatography
GC×GC	-	Comprehensive two-dimensional chromatograph/chromatography
MS	-	Mass spectrometer/spectrometry
TOFMS	-	Time-of-flight mass spectrometer/spectrometry
MS/MS	-	Tandem mass spectrometer/spectrometry
FID	-	Flame ionization detector/detection
m/z	-	Mass to charge ratio
LLE	-	Liquid-liquid extraction
DLLME	-	Dispersive liquid-liquid microextraction
SPME	-	Solid-phase microextraction
HS	-	Headspace
ELISA	-	Enzyme-linked immunosorbent assay
PCR	-	Polymerase chain reaction
DGGE	-	Denaturing gradient gel electrophoresis
DI	-	Direct immersion
DHS	-	Dynamic headspace (extraction)
CAR	-	Carboxen
DVB	-	Divinyl benzene
PEG	-	Polyethylene glycol
PA	-	Polyacrylate
TEH	-	Total extractable hydrocarbons
TPH	-	Total petroleum hydrocarbons
SPE	-	Solid-phase extraction
TD	-	Thermal desorption
MPS	-	MultiPurpose Sampler
S/N	-	Signal-to-noise ratio
PCE	-	Perchloroethylene
LOD	-	Limit of detection
LOL	-	Limit of linearity
CAD	-	Canadian dollars

EPA	-	Environmental Protection Agency
EI	-	Electron impact
R2022a	-	Software release: 2022, version a
TIC	-	Total ion current
EIC	-	Extracted ion current
MTBE	-	Methyl *tert*-butyl ether
PAH	-	Polycyclic aromatic hydrocarbons
DNAPL	-	Dense non-aqueous-phase liquid
COC	-	Contaminant of concern
MW	-	Molecular weight
VOC	-	Volatile organic compound
PDMS	-	Polydimethylsiloxane
HSPME	-	Headspace solid-phase microextraction
QqQ	-	Triple-quadrupole
CE	-	Collision energy
RO	-	Reverse osmosis
DCM	-	Dichloromethane
IS	-	Internal standard
DHS	-	Dynamic headspace
QC	-	Quality control
CIS	-	Cooled injection system
PTV	-	Programmable temperature vaporizing
SLH	-	Septumless head

List of Symbols

N_2	-	Nitrogen
He	-	Helium
H_2	-	Hydrogen
K	-	Partition coefficient
k	-	Retention factor
N	-	Number of theoretical plates
α	-	Selectivity factor
t_s	-	Time spent in the stationary phase
t_m	-	Time spent in the mobile phase / hold up time
V_s	-	Volume of the stationary phase
V_m	-	Volume of the mobile phase
t_R	-	Retention time
$t_{R,max}$	-	Maximum retention time
w	-	Width
$w_{1/2}$	-	Width at half height
w_b	-	Width at base
R_s	-	Resolution
n_p	-	Number of peaks (peak capacity)
H	-	Height equivalent to a theoretical plate
A	-	Eddy-diffusion parameter of particles in a chromatography column
B/μ	-	Diffusion parameter of particles in a chromatography column
C_s	-	Resistance to mass transfer of the stationary phase
C_m	-	Resistance to mass transfer of the mobile phase
μ	-	Linear velocity
q	-	Fraction of analyte remaining in aqueous solution after *n* extractions
m	-	Mass/molecular mass
V_1	-	Solution volume
V_2	-	Extraction solvent volume
P_M	-	Modulation period duration
y_{dl}	-	Detection limit signal

y_{bl}	-	Signal of a blank
MΩ	-	Mega-Ohms
t_0	-	Time zero (start time)
^1D	-	First dimension
^2D	-	Second dimension
(+)	-	Clockwise enantiomer
(-)	-	Counter-clockwise enantiomer
(±)	-	Racemic enantiomer mixture

CHAPTER 1: Introduction

1.1 Motivation

There is a conception in pop-culture and even within academia that if a discovery is important enough it will be utilized the world over. As technology advances, the industries connected to that technology will advance with it. Unfortunately, this has not been the case with environmental analysis, and specifically with routine environmental analysis. Advancements in extraction, separation and detection methods in the last 20 years make the information provided by analytical methods before then seem almost trivial [1,2]. Gas chromatography can now be replaced with comprehensive multidimensional gas chromatography, mass spectrometers and other detectors are orders of magnitude more sensitive and selective than they used to be, and there has been a great deal of work put into extraction technology allowing for lower than μg/L of environmental substances to be detected by modern instrumentation [1-4]. Despite all of this, these new technologies seem to remain within academia and struggle to become commonplace in routine analysis, with a staggering number of approved and accredited laboratory practices dating back to before the turn of the twenty-first century [5].

There are a number of barriers preventing new methods from being implemented in industry. Mostly related to costs and financial pressures. When a profit-seeking organization is looking to implement new technology, it is important to minimize financial risk [6]. If it is not clear that a method or technology will significantly improve workflows, productivity and profits it is less of a risk to continue to do things the way that they are already being done. For this reason, it is important to minimize capital investment required to implement new methods. Being able to use what they already have in new and interesting ways goes a long way toward bringing in new ideas. In this sense, if something new can retrofit or repurpose cheap or already available equipment and infrastructure it is better for business. Additionally, environmental analysis is a highly regulated industry. Environmental analyses tend to meet the requirements of government regulation and may be reluctant to expend resources to improve methods further that what is required of them. The reason for improvement then needs to be a selling point to clients. If they can be better informed they may be more likely to seek an analysis, especially if they have a genuine interest in the contents of a particular sample. Of course, there will be those who would prefer (often for financial reasons) that fewer analytes are detected rather than more, providing more pushback to innovation in the industry.

Another way that costs come into play is in the recruitment of staff who use instrumentation. When staff are being compensated for their work, especially on an hourly basis it benefits the organization to pay less per hour and to minimize the overall number of hours for any given workload and payout [7]. Because they are less costly to hire and retain, staff in contract laboratories may not have advanced degrees compared to other scientific professions meaning that they can be compensated less per hour or per annum. However, they may not have the technical expertise required to immediately understand emerging technologies and new instrumentation. Staff will also be expected to easily multitask and be trained on any methods that they will be using. It can be difficult to ensure that staff can effectively learn many complicated techniques, so it is more sensible to design and utilize methods that they can easily learn. Having fewer steps and using familiar equipment for multiple analyses can help to accomplish this. Minimizing equipment also has the positive side-effect of minimizing space, energy and materials used.

Above all else is performance. The information that a contract lab can give to their clients at a competitive rate and turn-around time is what will ultimately motivate clients to return to the lab for analyses that they need. They make themselves an asset when they can supply a result that is above and beyond what the client could have asked for. It is important to maximize the information about a certain analysis to allow the client to use the knowledge gained for their own means. Providing all this information as quickly and efficiently as possible means that repeatable, standardized, fast, and easy to use methods are crucial to providing a service that a client can trust.

The remainder of **Chapter 1** introduces some of the challenges faced by the routine analysis industry and provides background to some of the ways proposed to break into the industry and make an impact. The background behind gas chromatography and comprehensive two-dimensional gas chromatography are discussed, as well as extraction methods such as liquid-liquid extraction, dispersive liquid-liquid microextraction, solid-phase microextraction and dynamic headspace. **Chapter 2** explores a direct comparison between a commonly used liquid-liquid extraction used in industry and a benchtop dispersive liquid-liquid microextraction using comprehensive two-dimensional gas chromatography – mass spectrometry and flame ionization detection. **Chapter 3** does a different sort of comparison, determining which of a set of soil extractions would be most effective for the targeted extraction of a number of compound classes and non-targeted extraction. **Chapter 4** expands on liquid-liquid extraction and a method of relative quantification that can be applied in industrial analysis. **Chapter 5** details a long-term study using routine analysis designed to detect and characterize the breakdown of geosmin

by microbial cultures. It is able to help draw conclusions about how microbes are behaving in a sample of water and even provide information about what breakdown products are being produced. **Chapter 6** describes three different pilot studies. One of these studies used a routine-designed non-targeted liquid-liquid extraction to characterize the behaviour of water-resistant fabrics. The next of these studies used a routine-designed solid-phase microextraction to determine the presence of anesthetics and large organic molecules in water. The last of which was the foundation to select an extraction method for an agricultural fruit analysis that has been ongoing for over two years without major changes to the analytical method. **Chapter 7** draws conclusions about this thesis and proposes future ideas for the continuation of each of its component chapters.

1.2 Analytical Instrumentation

1.2.1 Gas Chromatography

The name “chromatography” originally comes from the process of separating colours, or chromas, of plant pigments and recording (-graphy) them on separation paper [8]. GC, in the simplest terms, is a method of separation for volatile (gaseous or easily converted to the gas phase) compounds of a mixture [9]. The necessary components of a gas chromatography system (Figure 1-1) are a method of sample introduction, an inert carrier gas, pressure control, a column with separation phase, an oven and a detector [10]. Sample introduction for GC can be by introducing a sample that is already in the gas phase, such as headspace volatiles or on-column injections of volatile compounds, or by volatilizing a sample into the gas phase using high temperature or other means [10-13]. Once in the gas phase a pressure controlled inert carrier gas takes the sample through a closed-pressure system to a detector without changing its chemical composition (i.e. by reacting with the sample). The inert gas used is usually helium, but nitrogen and hydrogen are also used [10,14].

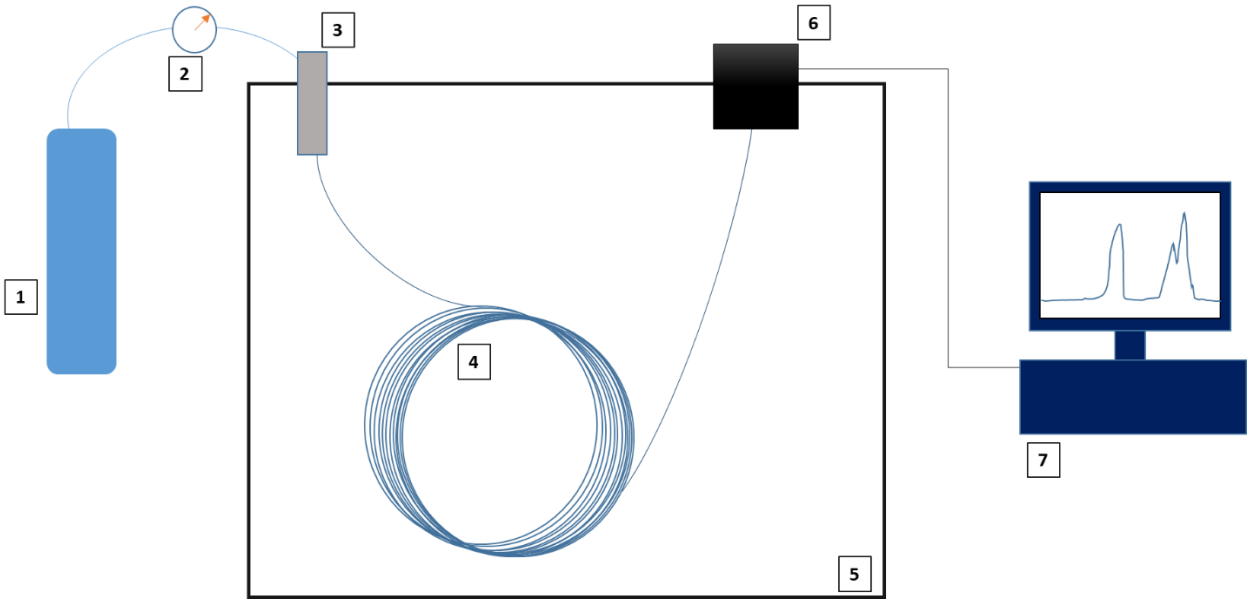


Figure 1-1. General schematic of a GC. (1) carrier gas supply with (2) pressure control; (3) sample introduction/inlet to a (4) wound capillary column in a (5) temperature-controlled oven; a (6) detector. All with communication to a (7) data collection system.

The most important parameters to consider for chromatography are partition coefficient (*K*), retention factor (*k*), the number of theoretical plates (*N*), and selectivity factor (α). These parameters are all used to determine how a separation will perform for a given analyte or comparison between analytes.

Equation 1-1 $$K = \frac{C_s}{C_m}$$

The partition coefficient, *K*, (also known as the distribution constant) represents the ratio between the concentration of analyte in the stationary phase of a separation column and the mobile phase (Equation 1-1) [15]. C_s is the concentration of sample component in the stationary phase; C_m is that in the mobile phase. As displayed in Figure 1-2, the stationary phase is the chemically active phase that performs the work to retain analyte. Analytes move through the column with the flow of carrier gas through the mobile phase.

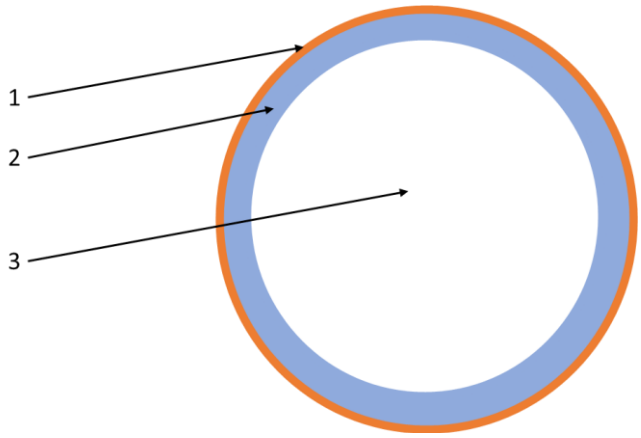


Figure 1-2. A cross-section of a capillary gas chromatography column. The (1) fused silica, (2) coated stationary phase, and (3) mobile phase are labeled.

k relates directly to *K* and is used to compare the travel rates of analytes through an analytical column. *k* does not depend on column geometry or volumetric flow rate. It does depend on analyte on column, temperature, mobile phase composition and stationary phase. It is defined as shown in Equation 1-2, where V_s is the volume of stationary phase and V_m is the volume of the mobile phase [16]. It can also be used chromatographically using Equation 1-3 [16]. t_r is retention time, accounting for the time an analyte spends combined in the stationary phase and mobile phase (t_s and t_m respectively).

Equation 1-2 $$k = \frac{K \cdot V_S}{V_m}$$

Equation 1-3 $$k = \frac{t_r - t_m}{t_m}$$

Selectivity factor (α) determines relative rates of travel of analytes through a column. Considering hypothetical analytes A and B, consider Equations 1-4 and 1-5. For each, analyte B is the more retained analyte. α can then be used as described in Equation 1-3 to substitute into Equation 1-5 to produce Equation 1-6. Equation 1-6, therefore, can be used to calculate α using a chromatogram [16].

Equation 1-4 $$\alpha = \frac{K_B}{K_A}$$

Equation 1-5 $$\alpha = \frac{k_B}{k_A}$$

Equation 1-6 $$\alpha = \frac{(t_R)_B - t_m}{(t_R)_A - t_m}$$

N represents the number of theoretical plates for a separation. Equation 1-7 shows how *N* can be obtained from a chromatographic separation. $w_{1/2}$ represents the width of a peak at half its maximum height. In the simplest terms, the greater the value of *N* the better resolution (Equation 1-8) will be [16].

Equation 1-7 $$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2$$

The goal of any chromatography system is ultimately to resolve peaks from different compounds. Resolution is a measure of the separation between adjacent peaks in a chromatogram. Resolution (R_s) can be expressed as shown in Equation 1-8 to relate the resolution of two compounds to *N*, *k*, and *α* directly [14]. Selectivity (represented by *α*) has the most significant influence on resolution compared to other parameters in the equation.

Equation 1-8 $$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k+1}$$

Optimization of chromatography system flows and dimensions can be calculated using the van Deemter equation (Equation 1-9) [10,14]. The van Deemter equation approximates the efficiency of a chromatographic column by accounting for band broadening. There are five components. *H*: resolving power; *A*: eddy-diffusion parameter accounting for flow through column packing; *B*: diffusion coefficient of particles within the column; *C*: sum of the resistance to mass transfer coefficients of the mobile (C_m) and stationary (C_s) phases; *μ*: linear velocity. Since modern gas chromatography almost exclusively uses capillary columns with no packing, the *A* term is reduced to zero.

Equation 1-9 $$H = A + \frac{B}{\mu} + (C_s + C_m) \cdot \mu$$

Columns range from of 0.1 mm in internal diameter at their narrowest to wide-bore columns up to 1 mm in internal diameter [17-20]. These columns are lined internally with a stationary phase made up of different chemistries that interact with components in a gaseous mixture in a predictable manner. Selectivity of a chromatography column is governed by this interaction. The more a compound interacts with the stationary phase the slower it will proceed through the column, therefore separating the components of a sample/mixture. To increase interaction with this stationary phase and thus increase separation of compounds, columns are made to be quite long. These lengths often range between 15 and 60 m but are shorter or longer depending on the application desired [17-20]. The column is coiled to fit inside an oven to control the temperature and separation speed of the contained gaseous mixture before connecting to a detector.

Different detectors can be connected to a GC column depending on the application, but the consistent theme of these detectors is an ability to quantitatively measure components of a mixture based on some detectable quality, such as compound mass, thermal conductivity, carbon content, halogen content, or other characteristic [14,21,22].

Chromatography has been in use since early in the 20th century with modern GC coming to fruition in the mid 20th century [23,24]. GC continues to be one of the most popular analytical techniques worldwide [10,24]. It has a perception of being quite robust, easy to troubleshoot and easy to use with a standardized method and relatively inexpensive to purchase upfront and to upkeep. Additionally, as with many techniques, high use in literature leads to more use from those seeking to build upon work and research being done. These factors combine to make GC an incredibly popular technique for environmental analysis. A significant portion of environmental contaminants are organic in nature and can in some way be volatilized and introduced to a GC system.

1.2.2 Comprehensive Two-Dimensional Gas Chromatography

Gas chromatography has been in use for most of the past century [25]. One issue that has always tended to plague conventional chromatography systems is the overlap between components and lack of resolution between compounds of similar chemistry. Regardless of the stationary phase chosen, in some situations it is almost impossible to avoid at least some co-elution of compounds. Additionally, constantly changing column designs to suit new samples can be time consuming and expensive. A major change was made to revolutionize GC by the conceptualization of a modulator in 1991 [26]. A modulator is designed to allow the direct connection of a second column to the chromatographic column [27,28]. This second column could then have very different (but complementary for separation) column chemistry. Comprehensive two-dimensional gas chromatography (GC×GC) was born. Figure 1-3 shows the key components that differ between a one-dimensional GC and a two-dimensional GC. Most importantly is the addition of a modulator. A modulator and the multiple separations in one analysis allow for higher peak capacity, better S/N ratio, and identification potential based on patterns available with the visualization of data. The most obvious benefits being less peak overlap and greater separation. GC×GC has been used in increasing frequency in recent years for detection and analysis and is only likely to become more popular as it reaches more and more into the mainstream [29,30].

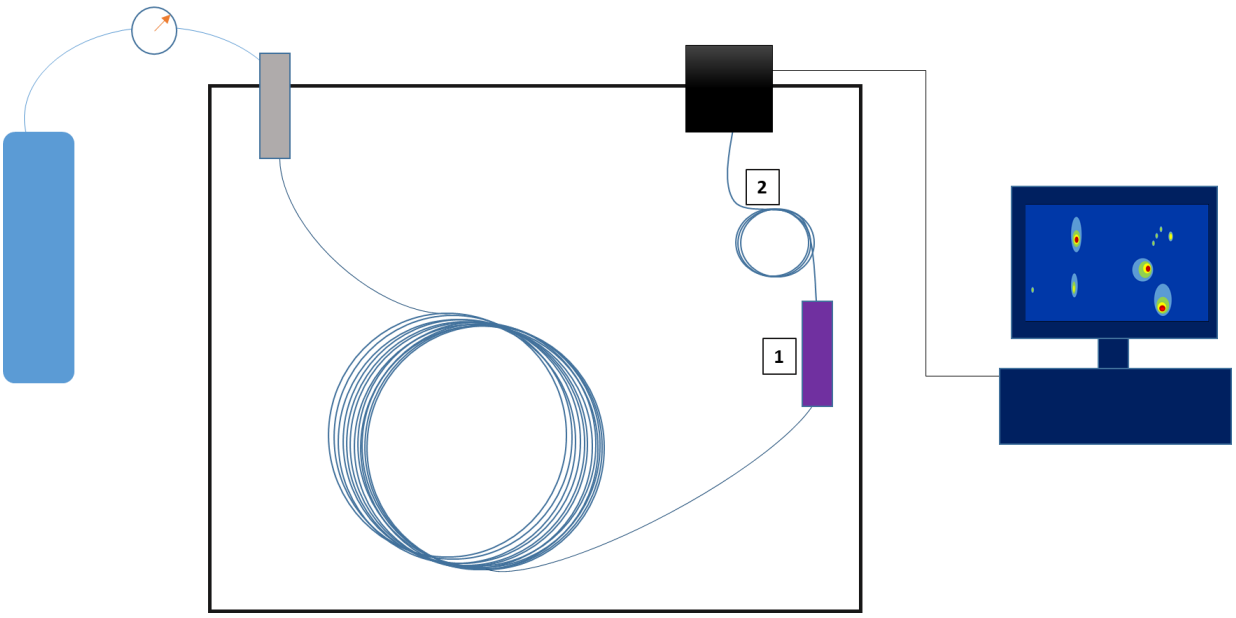


Figure 1-3. General schematic of a GC×GC. Setup is identical to a GC, aside from the use of a (1) modulator, modulator oven, and the functioning components of a modulator; connected to the first-dimension column is a (2) second-dimension column. Analysis software also has different requirements to handle and visualize two-dimensional separation data.

Figure 1-4 shows an example of how two-dimensional separation can benefit an analysis. First dimension retention time in Figure 1-4B is the same as the retention time in Figure 1-4A. The line plot consisting of x and y axes (retention time and intensity) has been replaced by a contour plot with retention times on both axes and a colour gradient bar acting as a z axis. Not only are the peaks that were once co-eluting now clearly resolved, but a number of small peaks that were not at all visible before in one-dimensional separation are now clear to see. There is also far more separation space (peak capacity) for additional peaks, whereas separation space in one dimension is far more limited. Equation 1-10 includes the formula for peak capacity in one-dimensional chromatography. Peak capacity is defined as n_p (number of perfectly spaced peaks that will fit in a chromatogram with a specified resolution R_s). t_M is the hold up time or the time it takes for an unretained analyte to exit the end of the column. $t_{r,max}$ is the maximum retention time for the separation, w_b is the width of a peak at the base, R_s is as defined in Equation 1-8. Two-dimensional separation peak capacity is the product of the first-dimension peak capacity and the second.

1-10
$$n_p = \frac{t_{r,max} - t_m}{w_b \cdot R_s}$$

In complex real samples, compounds with equivalent partitioning rates in the stationary phase such as isomers or other molecules with similar stationary phase interaction may co-elute in GC. Co-elution leads to misidentification or overestimation in quantification. Even when using detectors capable of identifying and differentiating between compounds such as mass spectrometers issues to separate and properly quantify co-elutions can still occur, often forcing researchers to go to great lengths to avoid co-elution [31].

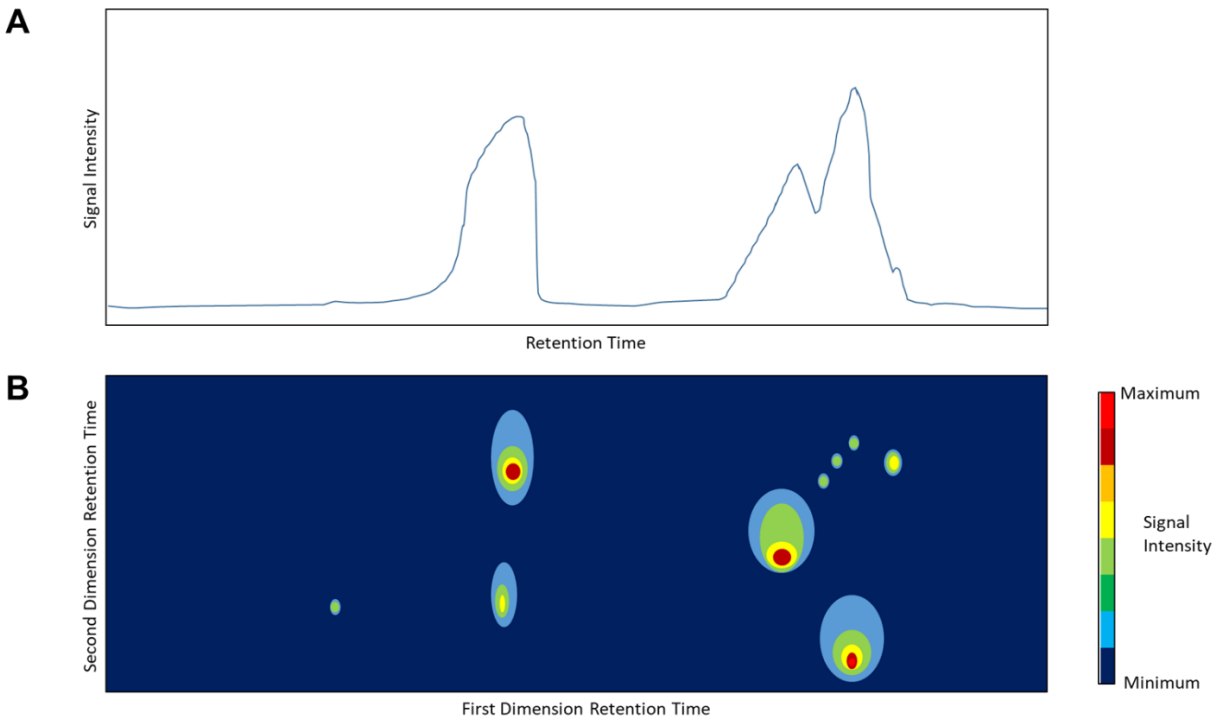


Figure 1-4. Theoretical injections of a sample using gas chromatography (A) and comprehensive two-dimensional gas chromatography (B).

There are multiple types of modulators available for GC×GC, but the fundamental principles are the same: get all of the sample coming from the first-dimension column to the second-dimension column while preserving the separation of the first dimension. It does this by trapping/accumulating primary column effluent from the first dimension and intermittently introducing the effluent collected onto the second dimension. Two examples are cryogenic and flow modulation. Cryogenic modulation utilizes alternating cold and hot jets of nitrogen gas on the separation column to drastically increase the retention factor, k, of particles and essentially stop them in the stationary phase momentarily at very low temperature [26]. Flow modulation uses a loop of inert column or tubing to trap volume from the first dimension before rapidly pushing it into the second dimension at a high flow [32,33]. Other

modulators use different mechanisms, but the foundational principle of "injecting" a concentrated pulse of the first-dimension separation onto the second dimension remains consistent.

Despite these benefits to its use and the relatively long time that it has been commercially available, GC×GC is still not as commonly used in industry as its advocates in academia believe it should be [34]. There is much more data available, which also means that the data is complicated relative to GC. There is a perception that GC×GC systems are comparatively more expensive to maintain and run based on additional costs for cryogen and additional flows and consumables. Due to its complexity relative to ^{1}D GC, SOPs and quality control steps are even more important to have in place to perform any regular maintenance and troubleshooting. As long as these are put in place, the benefits of GC×GC should make it invaluable for any environmental analysis lab.

1.2.3 Flame Ionization Detection

FID is often considered the simplest detector used with gas chromatography, and its reliability and quantification capacity for hydrocarbon-based compounds make it very popular both historically and currently in environmental analysis [4,35]. Flame ionization is especially common in the contract analysis industry due to its ease of use, reliability/robustness, low upfront cost, low maintenance cost, and overall quantification capability [35]. The detector functions by applying a high flow of air and hydrogen as fuel for a flame that rapidly burns and ionizes analytes comprising of -C-C- and -C-H bonds [10]. The more of these bonds that are present, the more intense the signal given off by the detector. The compounds that are detected best using flame ionization are volatile and semi-volatile organic compounds, meaning that GC is the ideal separation method for use with an FID. Even though FID does not provide any identification information of its own, the retention information provided by GC and GC×GC can help to identify components of a mixture, even when relatively complex [33,36].

FIDs have a very consistent linear range [10,36,37]. Signal is directly proportional to the number of susceptible carbon atoms entering the flame at any one time. This makes the detector incredibly useful for quantification of environmental samples, especially hydrocarbons. When paired with another detector, such as a mass spectrometer, flame ionization's linear range and quantification capabilities dovetail very well with the semi-quantification and identification capabilities of a mass spectrometer [36,38].

1.2.4 Mass Spectrometry

Mass spectrometry (MS) utilizes an ion source and mass analyzer to measure compound intensity based on the mass to charge ratio (m/z) of ions generated from the compound. Mass spectrometry is a very common detector that can be used in tandem with a number of other separation and analysis techniques [39]. The largest benefit of MS for all sample types is providing quantitative and qualitative information about compounds in a sample. The qualitative information provided by a mass spectrum makes MS very applicable and effective for a huge range of applications [39-43]. Despite all of this, MS still needs reliable separation, especially since it is not perfect at differentiating between all compounds of similar mass spectrum or fragmentation pattern [44]. TOFMS is by far the most common MS detector used for GC×GC for this reason [39-46]. Due to the chemical complexity of many environmental samples, the performance requirements of quantification, and the presence of a wide array of volatile and semi-volatile components, GC×GC-TOFMS is ideal for many environmental analyses [1,2,47,48].

1.2.5 Comprehensive Two-Dimensional Gas Chromatography – Time-of-Flight Mass Spectrometry and Flame Ionization Detection

When considering MS, one of its major drawbacks is a limit of linearity and response factor that is not consistent for all compounds at all ionization intensities. When considering FID, one of its major drawbacks is a lack of identification information to go along with its quantitative capacity. Using these two detectors in parallel would go a long way to overcoming the drawbacks of each by supplementing them with each other [36,38]. FIDs can have a linear range of $>10^7$ compared to mass spectrometers, which generally reach 10^5 [49]. Additional to the use of a modulator, the use of a microfluidic splitter at the end of the second-dimension column allows the diversion of flows to multiple destinations [38]. These destinations, as implied, can be separate detectors such as FID and MS. Carefully adjusting column lengths and diameters allows for samples to reach both detectors simultaneously. FID has the robust quantitation and linear range that MS does not have, and MS has the identification information that FID does not have. The detection limits of these detectors dovetail well for this purpose, since modern FIDs have an approximate detection limit of 2 pg/s, with MS detectors able to commonly reach 25 fg to 100 pg. Some state-of-the-art MS detectors are even able to reach sub-fg limits of detection [49].

1.3 Extraction Methods

1.3.1 Liquid-Liquid Extraction of Water Matrices

Liquid-liquid extraction (LLE) is the most common method for the extraction and analysis of volatiles and semi-volatiles for analysis by gas chromatography [33]. Simply relying on the value of the partition coefficient (K) between the solvent and sample to favourably extract a sample into the solvent [50]. Equations 1-11 and 1-12 show how K is calculated and how it relates to the fraction of analyte mass remaining in aqueous solution after *n* extractions (q). LLE and solvent extraction in general lend themselves well to analysis by GC. Liquid extractions tend to utilize organic solvents which are most suitable for injection and volatilization in a GC inlet and oven. These types of extractions tend to be easier than most to implement and adjust for use in different production scales and industries.

Equation 1-11
$$K = \frac{(1-q)(\frac{m}{V_2})}{q\cdot(\frac{m}{V_1})}$$

Equation 1-12
$$q^n = (\frac{V_1}{V_1+K\cdot V_2})^n$$

1.3.2 Dispersive Liquid-Liquid Microextraction of Water Matrices

Dispersive liquid-liquid microextraction (DLLME) was first proposed as a microextraction and preconcentration technique [51]. It involves the use of a dispersant solvent, which is soluble in both the extraction solvent (usually a non-polar organic solvent) and the sample (usually aqueous). The dispersant is aptly named because when rapidly injected into a sample it “disperses” throughout the sample volume, bringing the extraction solvent with it. This allows a small volume of extractant solvent to closely interact with the sample, allowing for more efficient interaction between particles in the sample and the extractant solvent without significant agitation and physical mixing being required. The solvents and sample can easily be separated from each other gravimetrically by centrifuging them, allowing lighter extraction solvents to float on top of a heavier water matrix or a heavier extraction solvent sink beneath a lighter water matrix. This makes the method very fast and easy to implement. These factors allow DLLME to be affordable, environmentally friendly, and analytically sensitive compared to other forms of liquid extraction [36].

The biggest factor making DLLME effective is how quickly equilibrium can be reached. The immediate dispersion of extractant throughout a sample solution using a dispersant allows the equilibrium time to be almost immediate. Equilibrium in an agitation method like LLE can often take up to hours depending

on the size of the sample being extracted. Low overall contact area between the solvent and sample greatly decreases how quickly equilibrium can establish.

1.3.3 Liquid Extraction of Solid Matrices

Liquid extractions, as the name implies, utilize liquid solvent to extract analytes from solid matrices. Liquid extraction methods are relatively simple to implement and are easily the most common method of extraction for semi-volatile organic compounds in industry. Agitation/shaking of the sample is often used to promote extraction of analytes into the organic matrix in the case of a cold-shake method [52]. The gold standard method in liquid extraction, however, is known as a Soxhlet extraction. In a Soxhlet extraction, solvent is continuously washed over the sample for a period of 16-24 h [53]. Figure 1-5 shows a Soxhlet apparatus and its common components. Soxhlet generally uses a large amount of solvent (i.e. 250 mL of hexane for a 10 g soil sample) which often requires post-extraction concentration by drying down and reconstituting into a smaller volume of solvent. Liquid extraction methods such as these aim to be exhaustive and one-time use. Because of the negatives associated with Soxhlet's large amount of solvent and long extraction time, it is the goal of other extraction methods analyzing solid matrices to approach the exhaustive extraction of a Soxhlet in much less time and with a much smaller amount of solvent. Many industrial analyses are moving away from Soxhlet extraction in favor of less exhaustive but faster and cheaper extraction methods such as cold shake [35].

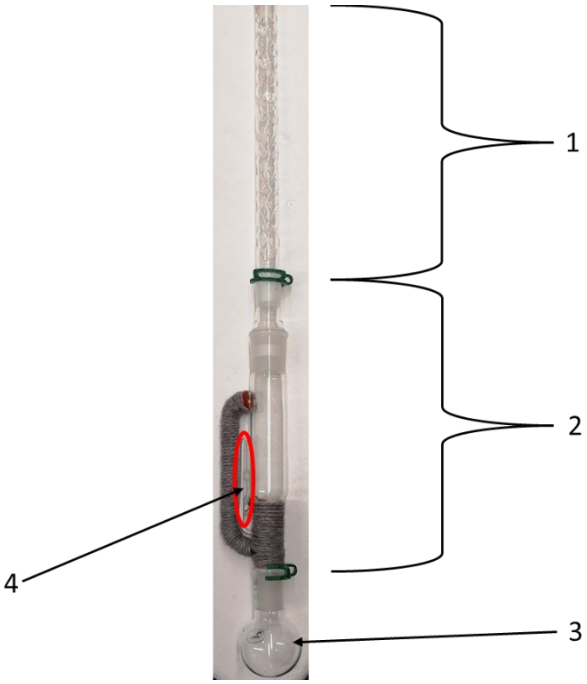


Figure 1-5. A Soxhlet apparatus and its primary components, (1) a condenser column, (2) the Soxhlet extractor, and (3) a round bottom flask. Once the volume of solvent in the extraction chamber reaches a level above the top of the (4) siphon loop the solvent drains from the extraction chamber into the solvent flask where it is boiled before being collected back into to extraction chamber by the condenser.

1.4 Sample Introduction Methods

1.4.1 Liquid Injection

Liquid injection utilizes the hot inlet of a GC to rapidly volatilize the components of an organic solvent in order to get it and its component analytes into the gas phase. Liquid injection generally follows extraction of a sample with an organic solvent; a small amount of solvent is transferred via syringe to a hot inlet (usually 200-300 ^{o}C). Injection volumes can vary between 0.1 µL up to 10 µL or more [54-56]. An advantage of liquid injection is the ability to solvent focus. This is a technique used when the temperature of the GC oven is maintained below the boiling point of the injected solvent, allowing it to condense and “focus” on the column before ramping up the oven temperature. This allows for sharper, better resolved peaks. One disadvantage of liquid injection, especially for non-targeted analysis, is the solvent itself. To solvent focus a splitless injection GC method (including the ones within this thesis) start their temperature program temperature below the boiling point of the solvent being injected.

Since the solvent contains an overwhelming amount of the solvent compound most peaks that fall within the retention time of the large solvent peak are buried and are often impossible to identify and

quantify. Sensitive detectors, such as MS, have functionality built in where one can set a solvent delay to turn off or decrease filament voltages in the time that a solvent is flowing through the detector. Use of a solvent delay is an essential way of preventing damage to a detector to avoid smashing it with countless particles of solvent. A mass spectrometer, for example, is prone to damage of its sensitive detector and filament, so using a delay on acquisition will greatly prolong the life of a detector, saving money and preventing instrument downtime in the long term. The only downside to the use of a solvent delay is that compounds that would normally coelute or elute earlier than the solvent aren't able to be analyzed. This is a price worth paying for labs that are dependent on minimizing downtime and reducing replacement equipment costs (which should be all labs).

1.4.2 Thermal Desorption

Thermal desorption has been gaining popularity in industry as a solvent-free method with simple application for solid environmental samples [1,57]. Aside from a high upfront cost compared to other extraction methods, mechanical maintenance, and additional gas usage, this method is very green compared to many methods that employ the use of solvents [58]. Unlike liquid injection, the temperature used to heat the sample is often less, with a chamber housing the sample or a sorbent containing a sample extract. An inert gas, usually nitrogen, flows over the sample while heating and brings the volatiles to a cold trap where they are bound at low temperature (and high k). The cold trap is then rapidly heated with a flow of carrier gas that takes the sample into the GC.

TD is very useful for solid samples with a large number of volatiles, especially when the volatiles would be masked from a detector by the presence of a solvent [59]. Extraction of samples and pre-treatment is often not necessary. A TD unit is often a separate instrument that is attached to the front of a GC through an interface, often requiring its own injection vessels and sample tubes. TD can be limited by the sample type being used. Water samples, for instance, cannot be directly placed in a thermal desorption tube. TD (particularly the one used for analysis in this thesis) relies on an internal cold trap, which can have a very high affinity for particular classes of compounds and those with very low volatility. This can make desorption of these compounds difficult, carrying over and contaminating the next sample in a sequence.

1.4.3 Solid-Phase Microextraction

Another solvent-less technique, solid-phase microextraction (SPME) uses a small fiber coated in stationary phase to extract a sample and thermally desorb into the inlet of a GC. Conveniently, it is designed in a way that only a specialized inlet liner is needed; otherwise, no changes need to be made to

a conventional GC setup. The temperature used to desorb the fiber often depends on manufacturer specifications and flows used are the same as liquid injection.

SPME can be used as a headspace (HS) extraction method or direct immersion (DI) in what is normally an aqueous sample, though many other interesting methods of application do exist [60]. Samples are wide-ranging and easily modified, meaning that sample types can range from the headspace of biological samples to DI of blood in the bloodstream, to the non-targeted analysis of organic contaminants in environmental soil and water [61-63]. Fibres can come in a range of thicknesses, from 7 μm up to 100 μm and can come in a number of different chemistries, namely polydimethyl siloxane (PDMS), carboxen (CAR), divinyl benzene (DVB) and polyacrylate (PA), as well as combinations of them [64]. Thicker fibres are compounds with higher volatilities and lower molecular weights, while thinner fibres are designed for heavier, less volatile compounds. There is, however, a lot of overlap between the volatilities and molecular weights that can be accommodated by different fibre sizes and chemistries. Generally speaking, PDMS fibres are selective for non-polar organic compounds, CAR fibres for non-targeted and broad applications, DVB fibres for aromatics and PA fibres for more polar organic compounds.

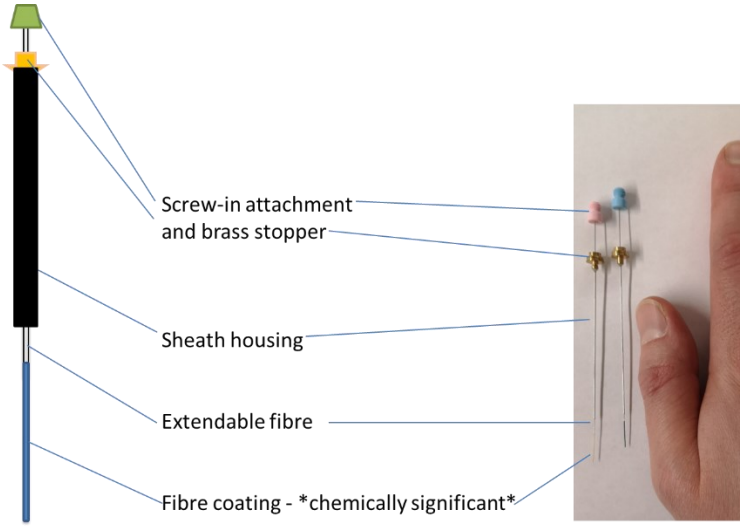


Figure 1-6. SPME fibre and its components. Scale shown compared to a human hand.

SPME is a method that extracts based on equilibria. As such, it is very dependent on the equilibrium between a sample and the fibre chemistry. The concentration of an analyte extracted by the fibre will depend largely on its kinetics as shown in Figure 1-7. For these reasons, SPME can be challenging to use for quantification and must have closely controlled extraction conditions including extraction time, temperature, and sample conditions (i.e. ionic strength and pH) [61].

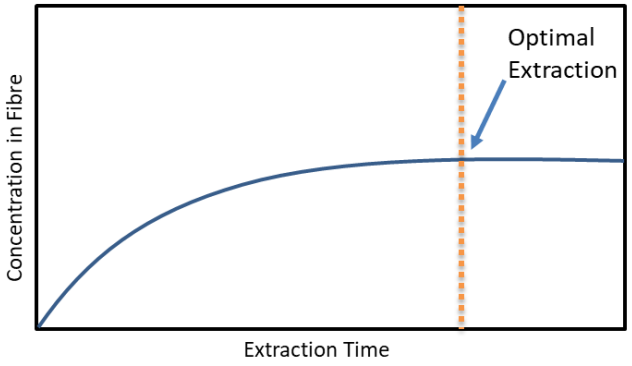


Figure 1-7. Extraction time profile of an analyte using an SPME fibre. Optimal extraction time generally corresponds with the point when the concentration of analyte in the fibre is in equilibrium with analyte in the sample.

1.5 Non-Targeted Analysis

The vast majority of environmental analyses are in some way targeted [35,65]. A targeted method has a particular compound or set of compounds that it is trying to identify, analyze and quantify. Total extractable hydrocarbon (TEH) methods, for example, are designed to extract hydrocarbons with 8-40

carbon atoms. Their analysis is tailored to these compounds and often don't aim to consider what else is in a sample. Many organic compounds are common contaminants in environmental samples and often come from industrial processes involving a wide variety of industries and applications. Many contaminants are monitored based on international and national guidelines and thresholds [35,65,66]. One set of examples are the derivatives of petroleum products, such as alcohols, ketones, nitriles, etc. which tend to partition more effectively in water, and the toxicology of which suggests that they may be more harmful than the petroleum compounds themselves. These are not traditionally targeted by routine environmental methods [66-69]. These compounds can be primary contaminants that are polluted into the environment, naturally occurring, or present due to biological processes, photodegradation, or other environmental factors [67,69]. Lack of information on their presence can therefore lead to underrepresentation of the levels of pollution based on traditional water analyses [70].

One way that new applications can be discovered is through non-targeted analysis. Particularly when doing exploratory research, beginning with a non-targeted approach can broaden the scope of a project and allow information to be gathered that otherwise would not have been [71,72]. Non-targeted extraction methods for GC therefore need to be able to accommodate a wide range of compounds. Generally, this is using a solvent or sorbent that is not specific in the types of compounds that it extracts from a sample [73].

1.6 Evaluation Metrics

When evaluating methods of their merits, there is more to consider than just their analytical figures of merit. Economic cost and environmental impact are examples of things that a contract laboratory would certainly care about. Unfortunately, both are relatively subjective, with analysis costs largely depending on the time taken to perform an analysis and even where and when it is being performed. It is important to consider every aspect of a method possible to avoid this subjectivity, including the cost of materials, reagents, staffing, transportation and waste disposal. This can be done for individual samples (single analysis) or batches of samples [36]. Economic analyses tend to lack nuance, mostly only considering face-value costs, but can still function to compare methods in a way that is useful to a would-be analyst or company.

Likewise, evaluating how environmentally friendly a method is can be difficult. A number of metrics have been designed to attempt to quantify how green a method can be [74]. There are a select few methods that have been designed specifically for analytical methods, which are most useful for evaluating technologies involved with extraction and separation [58,75]. These metrics help to make

evaluations of methods' environmental impact more objective. They also often use visual elements to make it not only obvious which of a set of compared methods is greenest, but also which aspects of an individual method are causing them to be most harmful to the environment. A metric called Analytical Eco-Scale designed by Galuska et al in 2012 was designed specifically for the assessment of analytical methods [76]. This metric, though not perfect for all analyses, provides a framework that can be used for the "greenness" evaluation of analytical methods and sample preparation.

1.7 Dissertation Objectives

Environmental samples are incredibly diverse. The information that can be gained from analyzing environmental samples can range from identification of a spill source to the discovery of new fossil fuel reserves, to the monitoring of industrial waste, and much more. Unfortunately, the industry specializing in environmental chemical analysis is lagging far behind what is being developed in academic circles. Reasons for this include the proverbial "if it works don't change it" mentality, economic considerations, and a simple lack of transferrable methodology from the most up-to-date academic methods and industry. The content of this thesis will aim to make methods and their implementation simple and effective when translated to an industrial or high throughput setting.

Chapter 2: Exploration of Extraction and Separation Techniques for Routine Trace Analysis of Organic Compounds in Water: Dispersive Liquid-Liquid Microextraction vs Liquid-Liquid Extraction[1]

2.1 Introduction

Many large-scale industrial analyses use single-phase (single extraction solvent) LLEs due to their simplicity and overall effectiveness for targeting specific compounds [6,7]. This can lead to compounds that are not targeted by the method not being adequately extracted and analyzed. LLEs tend to also have a significant environmental footprint because they use large volumes of both sample and solvent to achieve an effective extraction of an analyte from water [79-81]. Increasing the volume of sample will increase the amount of analyte extracted for analysis based on the partition coefficient between the sample and the solvent [82]. A comparatively large volume of organic solvent will increase contact between the solvent and insoluble sample matrix when the two are mixed thoroughly. However, it is not always possible to have the volume of sample necessary for sufficient extraction, and large volumes of solvent are undesirable for financial, environmental, and health / exposure reasons. When considering methods to be used at scale in industry, it is important to consider not only the analytical capabilities of a method but also how sustainable a method is from an environmental and cost perspective.

Non-targeted analyses of environmental pollutants in water using LLE have been recently demonstrated with a variety of compound classes and analyses [83-87]. The number of distinct chemical components in a sample mixture poses a significant challenge for chemical separation [88]. An LLE involving an organic solvent such as hexane acts as a benchmark for many methods, including by several labs with Canadian Association for Laboratory Accreditation certification [5]. Hexane is a significantly non-polar compound that does not lend itself well to global analysis outside of hydrocarbons and other non-polar compounds (which are accounted for by total environmental hydrocarbons - TEH and total petroleum hydrocarbons - TPH). A comparison of the advantages and disadvantages of extraction methods used in industry is provided in Table 2-1 [89,90].

[1] Portions of this chapter have been published as Johnson, T.J.; Armstrong, M.D.S.; de la Mata, A.P.; Harynuk, J.J. *J Chromatog Open.* 2022, 2, 100070.

Table 2-1: Extraction methods of semi-volatile organic compounds. The methods selected (aside from DLLME) are widely used in industry, with LLE being by far the most common of those listed.

*DLLME methods include those previously used to demonstrate the method [14,19-23].

Extraction Technique	Advantages	Disadvantages
Liquid-liquid extraction (LLE)	• Common in industry • Simple and reliable • Easily implemented and trained	• Large amount of solvent and waste • Low enrichment factor
Solid phase extraction (SPE)	• Low amount of solvent and waste • High enrichment factor • Resistant to emulsion	• Unstable extraction efficiency • Extraction column prone to physical blockage
Thermal desorption (TD)	• Small amount of sample needed • Solvent-free • High enrichment factor	• High upfront cost • Specific gas and equipment requirements • Limited to headspace
Dispersive liquid-liquid microextraction* (DLLME)	• Small amount of sample needed • Low solvent use/waste • High enrichment factor	• Specific sophisticated equipment required • Prone to matrix interferences • Uncommon in industry

DLLME was first proposed by Rezaee et al as a microextraction and preconcentration technique [91]. DLLME normally requires a specialized setup or instrument attachment for direct injection from the extracted medium to the instrument inlet [84,91-95]. We propose a DLLME method without this specialized instrumentation that can be adopted by any analytical laboratory. To achieve this, solvent and solution volumes are required to increase slightly. Despite this scale-up, DLLME still uses far less extraction solvent than a conventional LLE. The DLLME method presented has been designed to be easily implemented in any laboratory without high-cost or specialized equipment and instrumentation aside from a gas chromatograph. The implementation should be simple, and the method should provide

a fast alternative to many other types of extraction and pre-concentration which may be more time-consuming, particularly for individual samples [84,91,93].

Since the extracts of environmental samples often contain volatile organic compounds that originated from petroleum sources, a large percentage of analyses of hydrocarbons in the environment have utilized GC as a separation technique since well before the turn of the twenty-first century [77]. A more powerful separation technique, such as GC×GC is being used with increasing frequency for environmental detection and analysis, while effective means of detection and characterization, such as TOFMS, have also been used in increasing frequency [79,84,96,97]. Due to the chemical complexity of many environmental samples, the performance requirements of quantification, and the presence of a wide array of volatile and semi-volatile components, GC×GC-TOFMS is ideal for many environmental analyses [96].

Maximizing the number of detectable compounds is important for methods being used to analyze environmental contaminants. Consequently, using universal solvents and extraction systems, with separations that maximize peak capacity coupled to sensitive detection systems will provide the best opportunity to obtain a holistic view of organic constituents in environmental water samples. Analytically, this approach strives to detect alkanes in the C10-C40 region, PAHs, various hydrocarbon derivatives, breakdown species, and other contaminants that may also be present in water samples. In this study, we compare an LLE method that is commonly used in contract laboratories with a new DLLME protocol, while demonstrating the capability of the new method to also provide reasonable quantitative performance for non-target compounds detected in the sample.

2.2 Materials and Methods

2.2.1 Solvents and Standards

o-terphenyl (K&K Laboratories Inc., Carlsbad, CA, USA) was used as a surrogate. Naphthalene, phenanthrene, fluorene (Matheson-Coleman-Bell, Gardena, CA, USA), dodecane, pentadecane, octadecane, eicosane, methyl laurate, pyrene, 1-methyl-naphthalene, 2,7-dimethylnaphthalene, 1-methylphenanthrene, benz[a]anthracene, 2-tetradecanol, 7,12-dimethylbenzanthracene, and 2-tetradecene-4-ol (Millipore-Sigma Co., St. Louis, MO, USA) were chosen as representative petroleum compounds of interest. To this set, we added decanal (Pfaltz & Bauer, Waterbury, CT, USA), 2-heptadecanone, 1-naphthaldehyde (Eastman Chemical Co., Kingsport, TN, USA), 1-naphthol, 4-chloro-1,1'-biphenyl, 7,12-benz[*a*]anthracene-dione (Millipore-Sigma Co., St. Louis, MO, USA), 1-chlorooctane

(Tetrochem Laboratories, Edmonton, AB, Canada), and 4,4'-dichloro-1,1'-biphenyl (Chemicals Procurement Laboratories Inc., College Point, NY, USA). Adamantane (Millipore-Sigma Co., St. Louis, MO, USA) represented a petroleum fingerprint compound. Solvents used included dichloromethane and methanol as liquid injection wash solvents, tetrachloroethylene as an extraction solvent (all Millipore-Sigma Co., St. Louis, MO, USA), *n*-hexane (Fisher Scientific, Ottawa, ON, Canada) as an extraction solvent, and acetone (Fisher Scientific, Ottawa, ON, Canada) as a dispersant. Internal standards used were naphthalene-d8 (Isotec, Canton, GA, USA) and heptadecane-d36 (CDN Isotopes, Pointe-Claire, QC, Canada). Water was purified to 18 MΩ using a PURELAB Flex 2 Polisher (ELGA, High Wycombe, United Kingdom).

2.2.2 Instrumentation

Portions of the study that involved limits of detection for the extraction were performed on cryo-GC×GC-TOFMS since unlike flow-GC×GC-TOFMS there is no potential for loss of sample out a modulator bleed line and there is no splitter to reduce the high second-dimension flow heading to the detector. Subsequent portions of the study were conducted using flow-GC×GC-TOFMS for its practicality in industry since it does not require additional cryogen, and it is easier to maintain for routine analysis in an industrial setting.

2.2.2.1 Cryogenic GC×GC-TOFMS

LOD analyses were performed using a LECO Pegasus 4D GC×GC-TOFMS equipped with a four-jet, dual-stage modulator (LECO, St. Joseph, MI, USA). The ^{1}D column was a 31.0 m × 0.25 mm; 0.25 µm film thickness Restek Rtx-5 (Chromatographic Specialties, Brockville, ON, Canada). The ^{2}D column was a 1.2m × 0.25 mm; 0.25 µm film thickness Restek Rtx-200 (Chromatographic Specialties, Brockville, ON, Canada). The modulation period was 6 s, with hot and cold pulses of 1.2 and 1.8 s, respectively. The oven program started at 90 °C (3 min hold), 3 °C/min to 150 °C, 10 °C/min to 300 °C (10 min hold). The second-dimension column was programmed to have an offset of +5 °C relative to the primary oven, and the modulator was programmed to have an offset of +15 °C relative to the primary oven. The carrier gas was helium (5.0 grade, Linde, Edmonton, AB, Canada) at a constant flow of 1 mL/min. A GERSTEL MultiPurpose (MPS) Autosampler (GERSTEL GmbH & Co., Mülheim an der Ruhr, Germany) was used for injecting samples. Injection volume was 1 µL. The inlet was a CIS-4 PTV (GERSTEL GmbH & Co., Mülheim an der Ruhr, Germany) and used in splitless mode at 300 °C. The mass spectrometer was operated with an electron impact voltage offset of 200 and electron energy of -70 eV. A mass range of 40-600 m/z was collected at 200 Hz.

2.2.2.2 Flow GC×GC-TOFMS

Quantitative and real sample analyses were performed using an Agilent 7890A chromatograph (Agilent Technologies, Santa Clara, CA, USA) fitted with an INSIGHT flow modulator operated in reverse fill-flush mode (SepSolve Analytical, Waterloo, ON, Canada). The ^{1}D column was a 30.0 m × 0.25 mm; 1 µm film thickness Restek Rtx-5 (Chromatographic Specialties, Brockville, ON, Canada). The ^{2}D columns explored were both 5.0 m × 0.25 mm; Rtx-200 phase with thicknesses of 0.25 and 1.0 µm (Chromatographic Specialties, Brockville, ON, Canada).

A 7683 series auto-injector (Agilent Technologies, Santa Clara, CA, USA) was used to inject samples (1 µL) into a split-splitless injector used in splitless mode and set to 300 °C. 5.0 grade He (Linde Gas & Equipment Inc., Burr Ridge, IL, USA) was used as the carrier gas at 0.73 mL/min in ^{1}D and 15.4 mL/min in ^{2}D. The modulation period was set to 2.3 s (flush 230 ms). A 2.8 m × 0.1 mm deactivated fused silica bleed line was used for the modulator. The oven program was 90 °C (2 min hold), 7 °C/min to 300 °C (10 min hold). After the ^{2}D column a two-way purged splitter was used to divide flow between an FID and mass spectrometer. The FID was operated at 300 °C with flows of 40 mL/min H_2, 250 mL/min air, and 20 mL/min makeup gas (He). The mass spectrometer was a BenchTOF-Select mass spectrometer (Markes International Ltd, Bridgend, UK), operated in tandem electron ionization mode (-12 eV and -70 eV) at 100 spectra/s in each mode. Filament voltage was set to 1.7 V, and a mass range of 40-600 m/z was selected.

2.2.3 Data Processing and Statistical Analysis

2.2.3.1 ChromaTOF® for Cryogenic GC×GC-TOFMS

Data was processed using ChromaTOF® version 4.71.0.0 (LECO, St. Joseph, MI, USA). The baseline offset was set to 0.8 and the expected peak widths throughout the entire chromatographic run were set to 12 s for the first dimension and 0.15 s for the second dimension. The peak-finding threshold of signal to noise (S/N) was set to 50:1 with the minimum S/N ratio for sub-peaks to be retained set at 10. Mass spectra of chromatographic peaks were searched against NIST-MS and Wiley Libraries. The Statistical Compare feature of ChromaTOF® aligned the peak tables across standard runs and ensured replicate ions were chosen to quantify a given peak throughout the data set. Tolerances for retention time shift were ± 5 modulation periods (P_M = 6.0 s) in the first dimension, and 0.2 s for the second dimension. The minimum similarity for the mass spectral match to combine sub-peaks was set at 675/1000 using m/z values from 40 to 600.

2.2.3.2 ChromSpace® for Flow GC×GC-TOFMS

Data was processed using ChromSpace® version 2.0.2 (Markes International Ltd., Llantrisant, UK). Three different processing methods were used, one for each of the three methods of detection (-70 eV, -12 eV, and FID). The -70 eV EI TOFMS processing method utilized a curve fitting algorithm with 5 pseudo-Gaussian smoothing points. A baseline offset of 0.75 s was used for the second dimension. A minimum peak area was set at a value of 5000 counts^2, with a minimum height of 2500 counts and width of 0.1 minutes. NISTMS search 2.0 and Wiley 2008 databases were used to compare with mass spectral data obtained from individual peaks. A match of 675/1000 was considered a tentative match for the identity of compounds in non-targeted/relative quantitative analysis. The soft ionization -12 eV EI TOFMS processing method also utilized a curve fitting algorithm with 5 pseudo-Gaussian smoothing points with an identical baseline offset of 0.75 s in the second dimension. A minimum area of 1000 counts^2 and minimum height of 350 counts was considered for the sizes of peaks. The width parameter was set at 0.001 minutes. The FID processing method, like the TOFMS methods used a curve fitting algorithm with 5 pseudo-Gaussian smoothing points and a baseline offset of 0.75 s for the second dimension. An area of 8000 counts^2, height of 2000 counts, and width of 0.01 minutes were considered for minimum peak dimensions. Peak identities relied on mass spectral information obtained simultaneously by the -70 eV (for mass spectral library identity) and -12 eV (for molecular ion) EI data and retention indices of target compounds.

2.2.3.3 Limit of Detection and Quantitation

Signal detection limit of standards used the equation $y_{dl} = 3 \times s + y_{bl}$, where s is the standard deviation of the lowest detected standard's signal, y_{bl} is the signal of a blank, and y_{dl} is the minimum detectable signal [98]. Peak area thresholds were reduced very low (100 count^2 area, 100 count height, 0.001 minute width) in order to detect peaks with area in blanks. These were calculated with $n = 7$ replicates.

2.2.4 Sample Collection, Storage, and Preparation

2.2.4.1 Serial Dilution Standard Preparation

1 mL of a standard mix containing the maximum water-soluble concentrations of all 21 calibration compounds was added to a 2 L separatory funnel filled with 2.0 L of 18 MΩ·cm water. After thorough mixing, 3 × 230 mL aliquots of this solution were used for LLE, while 3 × 20-mL aliquots were used for the DLLME method. 1 L of the remaining solution was diluted in 18 MΩ·cm water to a final volume of 2 L. This process was repeated for all subsequent dilutions. In total, 11 dilutions were performed to

produce the calibration curves used for standards and the limits of detection for each extraction method.

2.2.4.2 Sampling for Real Sample Analysis

For flow-modulated GC×GC-TOFMS, 27 real samples were collected from surface waters in the Edmonton, Alberta, Canada area (Figure 2-1). Sampling was done using 250 mL amber sample bottles (Fisher Scientific, Ottawa, ON, Canada) filled without headspace. Samples were collected in duplicate and refrigerated at 7 ^{o}C until the day of extraction. Before being opened and extracted, samples were left at room temperature for 1 h. 20 mL of each sample was measured using a 20 mL volumetric pipette (Fisher Scientific, Ottawa, ON, Canada) and transferred to a 45 mL glass centrifuge tube (Fisher Scientific, Ottawa, ON, Canada) for analysis by DLLME. The remaining volume (230 mL) was kept in the 250 mL bottle to be used for LLE. For cryogenic-modulated GC×GC-TOFMS, 12 real samples with anticipated presence of target compounds, including an array of hydrocarbon compounds and PAHs were supplied by an industrial partner. Each sample received was in a 250 mL amber glass bottle without headspace and was distributed by volume as described previously. Details of sampling locations and conditions are included in Appendix A.

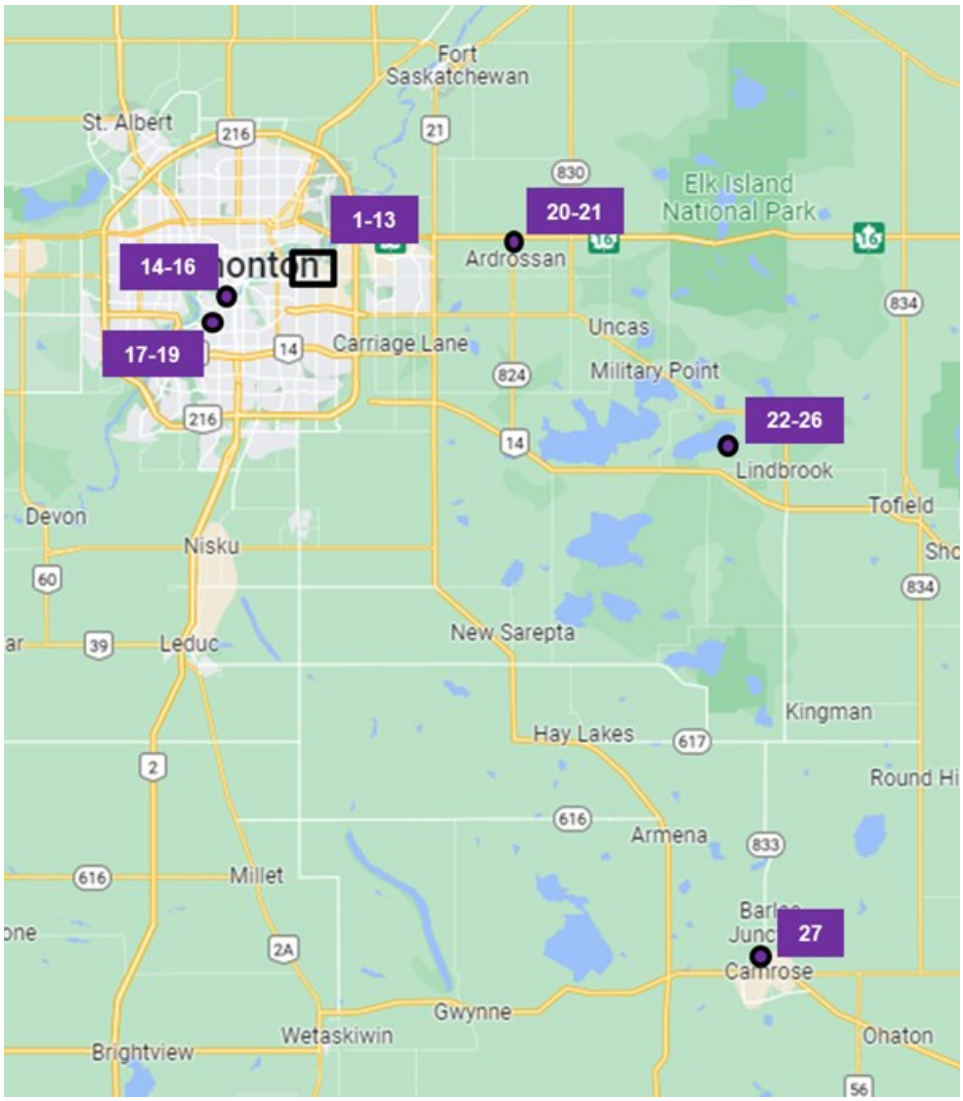


Figure 2-1. Sampling locations for real samples used in flow-GC×GC-TOFMS analysis. Each purple box covers an area where samples were collected. Details of sampling locations and chromatograms for each are included in Appendix A.

2.2.5 Extractions

Each extraction was performed in triplicate. Before adding solvents, 20 µL of 10 000 µg/mL *o*-terphenyl in acetone was added to each sample as a surrogate using a 50-µL glass syringe (Chromatographic Specialties, Brockville, ON, Canada). Heptadecane-d36 was incorporated into hexane (4.0 µg/mL) and perchloroethylene (PCE) (4.5 µg/mL) solutions for LLE and DLLME analyses, respectively, as additional surrogates for analysis of real samples.

2.2.5.1 LLE Extraction Procedure

Figure 2-2A shows the LLE extraction protocol which was modeled after a method used in a contract routine environmental analysis laboratory (AGAT Industries, Calgary, AB). A 5-mL glass syringe (Chromatographic Specialties, Brockville, ON) was used to add 5 mL of hexane to each sample. The solution was then mixed at 250 rpm for 15 min on a modified vortex mixer (BenchMixer V2, Benchmark Scientific, Edison, NJ) with an aluminum plate and belt strap to hold bottles in place while shaking. Next, the solution was sonicated (3510 ultrasonic cleaner; Marshall Scientific, Hampton, NH) for 5 min to eliminate emulsions. 18 MΩ·cm water was added to the bottle until the floating solvent reached the neck. Finally, a Pasteur pipette (Fisher Scientific, Ottawa, ON) was used to transfer a portion of the top hexane layer into a 2 mL GC vial (Chromatographic Specialties, Brockville, ON) for analysis.

2.2.5.2 DLLME Optimization

A scaled-up modification of the DLLME extraction volumes used by Rezaee et al, 2006 was used as a starting point for DLLME volumes [91]. A 20 mL sample volume was chosen to achieve an approximate 10-fold decrease in sample volume compared to the industry method volume of 230 mL. The smallest possible volume of extractant that could be successfully drawn from the bottom of a 50 mL centrifuge tube was 20 µL. Varying volumes of extractant up to 50 µL were tested along with varying dispersant volumes from 1 to 3 mL.

2.2.5.3. Optimized DLLME Extraction Procedure

Figure 2-2B presents the optimized DLLME extraction procedure (see section 3.1 for optimization). 20-mL aliquots of water sample were measured and collected in 45-mL glass centrifuge tubes and extracted using a mixture of PCE and acetone in a ratio of 1:40 by volume; this mixture was prepared in a large batch and swirled vigorously until homogeneous. 2 mL of this mixture was rapidly injected to the sample matrix using a 5-mL glass syringe (Chromatographic Specialties, Brockville, ON). The injection produced an immediate cloudy solution. This solution was next centrifuged at 3650 rpm for 2 min (International Equipment Company International, Needham, MA). A 100 µL gas-tight syringe with a flat-tipped needle (Chromatographic Specialties, Brockville, ON) was then used to carefully draw up 50 µL of the bottom PCE layer of solution and transfer it to a GC vial with a 300-µL glass insert (Chromatographic Specialties, Brockville, ON). This vial was used for injection on GC×GC-TOFMS and GC×GC-TOFMS/FID.

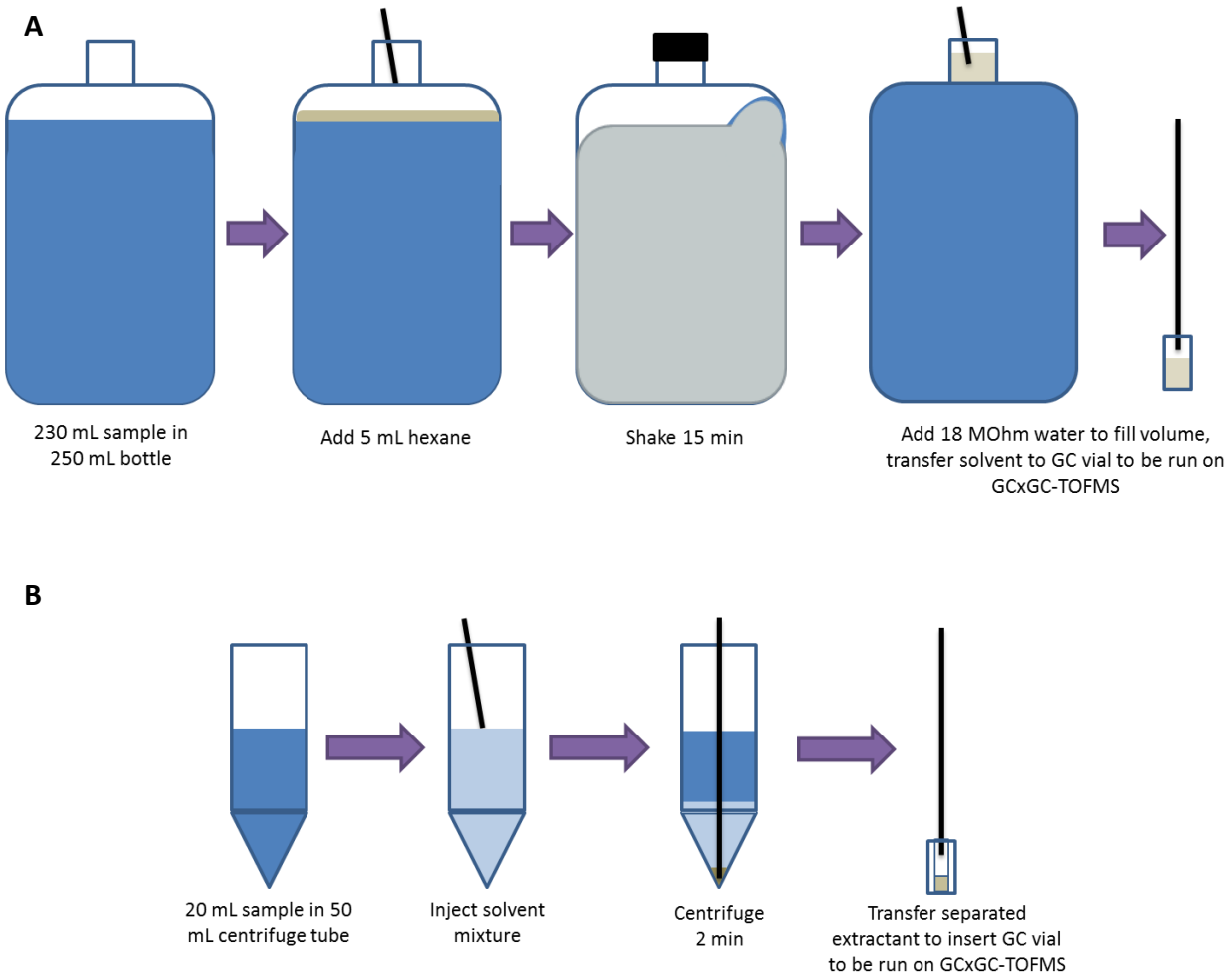


Figure 2-2. Extraction overviews. (A) Industry LLE protocol, (B) Developed DLLME protocol.

2.2.6 Cost and Greenness Analysis

Cost and greenness analyses were performed for both the LLE and DLLME methods to compare them using metrics beyond analytical performance.

Greenness evaluations were performed using a metric designed by Galuszka et al, 2012. Indicated in the table are “penalty points” given for each of the given descriptors. The “total” is calculated by subtracting the penalty points from 100; the closer a method gets to a score of 100 the more environmentally friendly the method is considered. Analytical Eco-Scale was chosen for this comparison due to its relevance to analytical preparation methods.

Extraction time costs were calculated based on the expected wage of a technician working in a CALA certified lab [99] divided by how long it would take to analyze a small batch of 6 samples. For the cost of shipping, the amount was recorded to ship a single sample and a batch of 50 samples. Solvent costs

were taken from the Millipore-Sigma website [100]. The cost of each solvent used per sample was calculated using the volume of solvent and how much it would cost to use for each based on volume needed. Purchasable consumables were calculated based on the real acquisition cost of equipment used for each analysis during the study. Main consumables were glassware (Boston-round bottles vs centrifuge tubes), Pasteur pipettes, and glass syringes. Waste disposal costs were calculated based on quoted amounts by a product manager at University of Alberta Environment Health and Safety, where waste disposal is done in bulk. The cost difference between disposing of halogenated solvents compared to non-halogenated organic solvents was negligible at either of the scales used.

2.3. Results and Discussion

2.3.1 Selection of Dispersant and Extractant Volumes

The extraction/dispersion solvent ratio was selected using results shown in Figures 2-3 and 2-4. A total of six extraction ratios were tested: 1:60, 1:50, 1:40, 1:30, and 1:20. The optimal extractant volume was in part selected from three ratios of 50 µL : 3 mL, 30 µL : 1.5 mL, and 20 µL : 1 mL of PCE : acetone. These ratios (1:60, 1:50 and 1:50, respectively) were initially chosen based on a scaled-up version of the method by Rezaee, et al. [89] and were modified and selected based on quantification results. Each was run in triplicate. Figure 2-3 shows results for the five compounds tested (naphthalene, dodecane, 2,7-dimethylnaphthalene, tetradecanol, and decanal) that were used to represent the classes of PAH, alkane, alkylated PAH, alcohol, and ketone/aldehyde respectively. Mixtures with smaller volumes of PCE (30 µL and 20 µL) tended to have higher recoveries of analytes. However, these smaller volumes became progressively more difficult to recover from sample matrices after extraction and had large errors in measured values as a result, and even resulted in them being comparable in performance to the larger volume extractions in some cases. The optimal extractant volume, which presented the smallest practical volume with the smallest standard deviations, was 50 µL. Steps were taken to minimize the amount of solvent that could be used for the analysis with this extractant volume. Smaller acetone : PCE ratios would allow for much less solvent to be used, so 50 µL : 2 mL, 50 µL : 1.5 mL, 50 µL : 1 mL (1:40, 1:30, and 1:20) were tested to improve upon the 50 µL : 3 mL ratio, which used more acetone than desired when considering solvent waste of the method. A variety of compounds were selected to examine the responses of analytes using these three new ratios. The smallest dispersant volumes that were able to properly disperse and allow for PCE to be recovered after centrifugation were 2 mL and 1.5 mL. 1 mL caused beading of PCE that did not properly disperse within solution before centrifugation. The recovery difference between 2 mL and 1.5 mL is shown in Figure 2-4. Compounds were spiked to

the standard mixture in varying amounts, covering different concentrations (ranging from 0.01 mg/L to 1 mg/L) for compounds of the same class. Triplicate extractions showed that a 2 mL dispersant volume was consistently more effective when extracting target analytes compared to a 1.5 mL volume. The optimal ratio used for further analyses was therefore 50 µL extractant with 2 mL of dispersant.

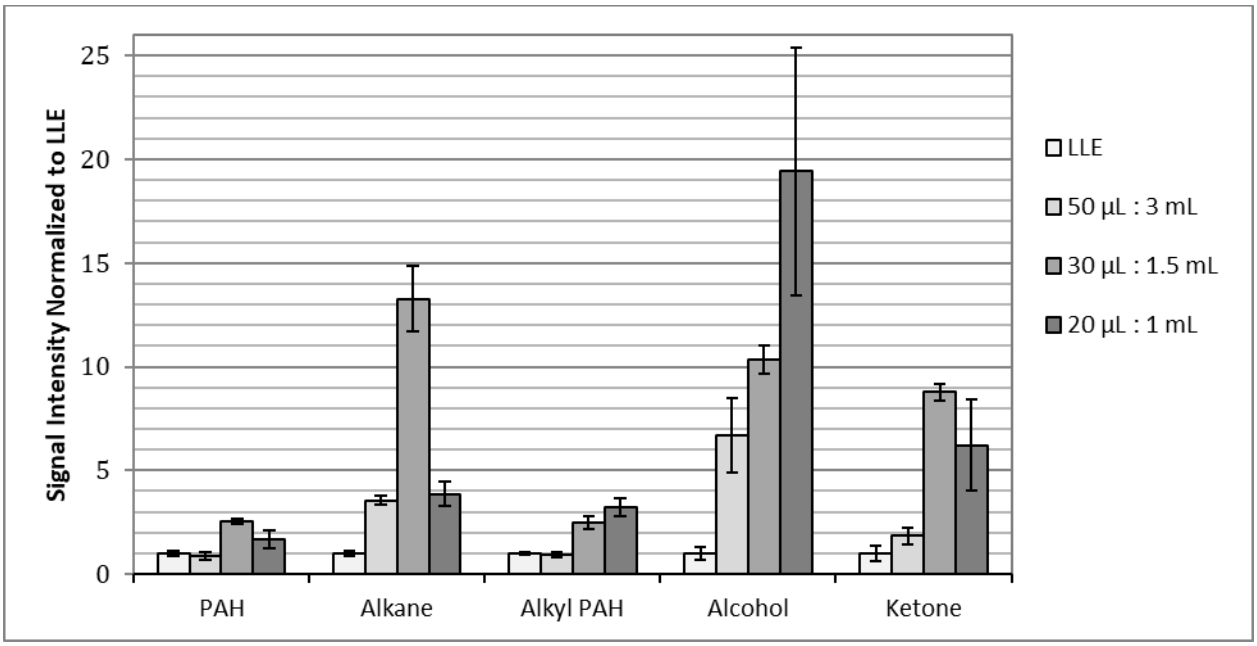


Figure 2-3. Peak intensities for extraction/dispersion solvent ratios. Intensities are normalized to give LLE a value of "1.0" for comparison. Error bars represent one standard deviation (n = 3). Representative compounds are naphthalene, dodecane, 2,7-dimethylnaphthalene, tetradecanol, and decanal, respectively. Smaller volumes of extractant improved extraction, but were much more difficult to collect for analysis.

Different extraction times were also tested for DLLME. Immediate, 5 and 10 minute intervals were initially tested to determine whether equilibrium was being reached before centrifugation at each given time point. There was no discernable change in spike recovery between the different time points. As a result, samples were all centrifuged immediately after injection with solvent mixtures.

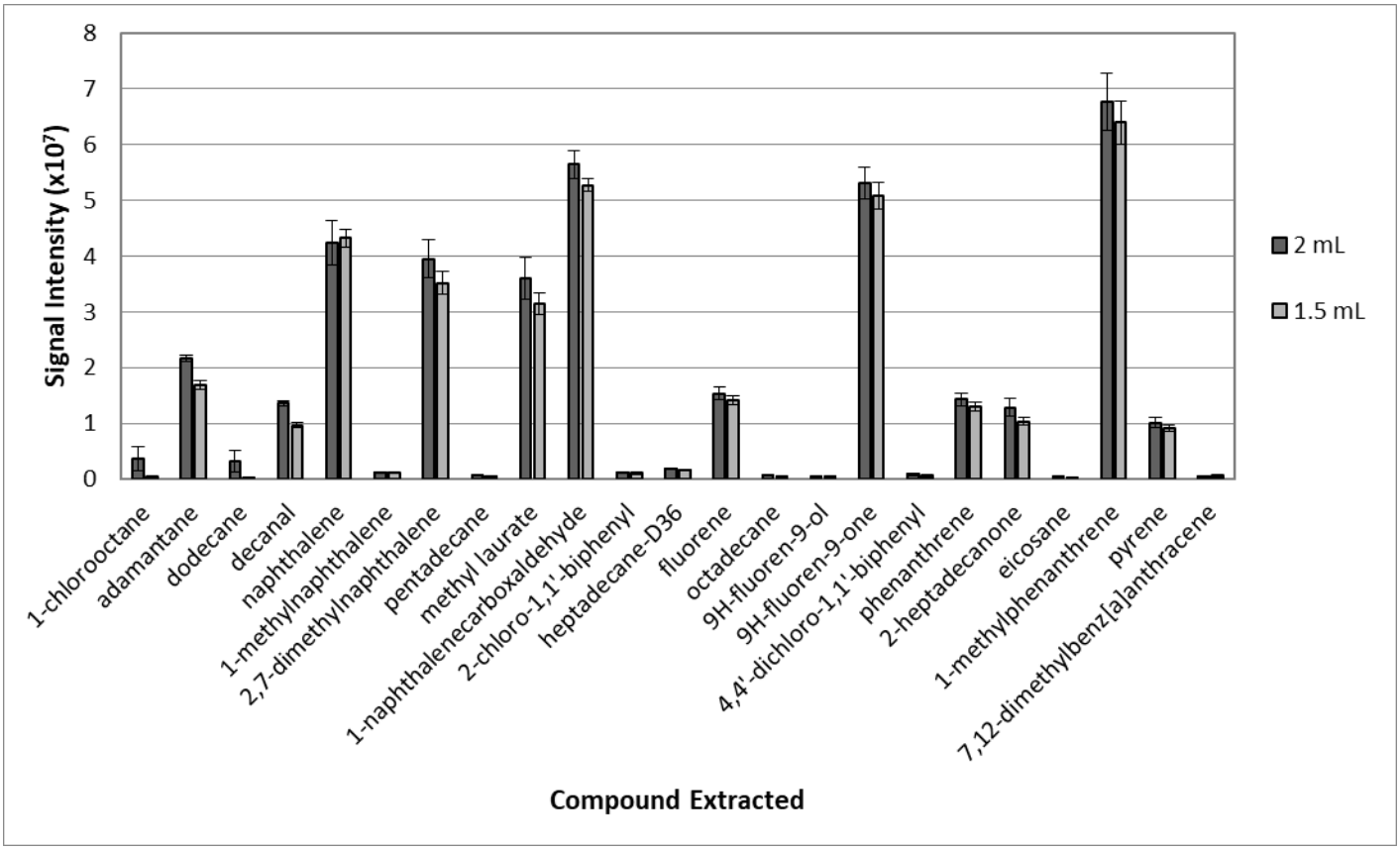


Figure 2-4. Peak intensities for extractions using the different dispersant volumes with 50 µL extractant volume. Error bars represent one standard deviation (n = 3).

2.3.2 Extraction and Method Characterization Using Cryogenic GC×GC-TOFMS

A standard solution containing 21 compounds (Section 2.1) was used to compare the LLE and DLLME extraction methods' detection limits. Figure 2-5 shows a comparison of extracted compound intensities using a 40 µg/L spiked sample with identical scales on the z-axis. Compound 16 (*o*-terphenyl) was used as a surrogate for the LLE method, so has a greatly inflated peak intensity. As can be seen from Figure 2-5B, the DLLME method concentrated analytes sufficiently so that all 21 spiked compounds were detectable, while only four analytes were detectable with the LLE method. DLLME used less than one tenth the sample solution and one one-hundredth the extraction solvent when compared to the LLE method. The chromatograms in Figure 2-5 are generated using mass channels 57, 74, 68, 81, 128, 178, and 202. These were selected based on common m/z ratios of interest for the compounds in the mixture.

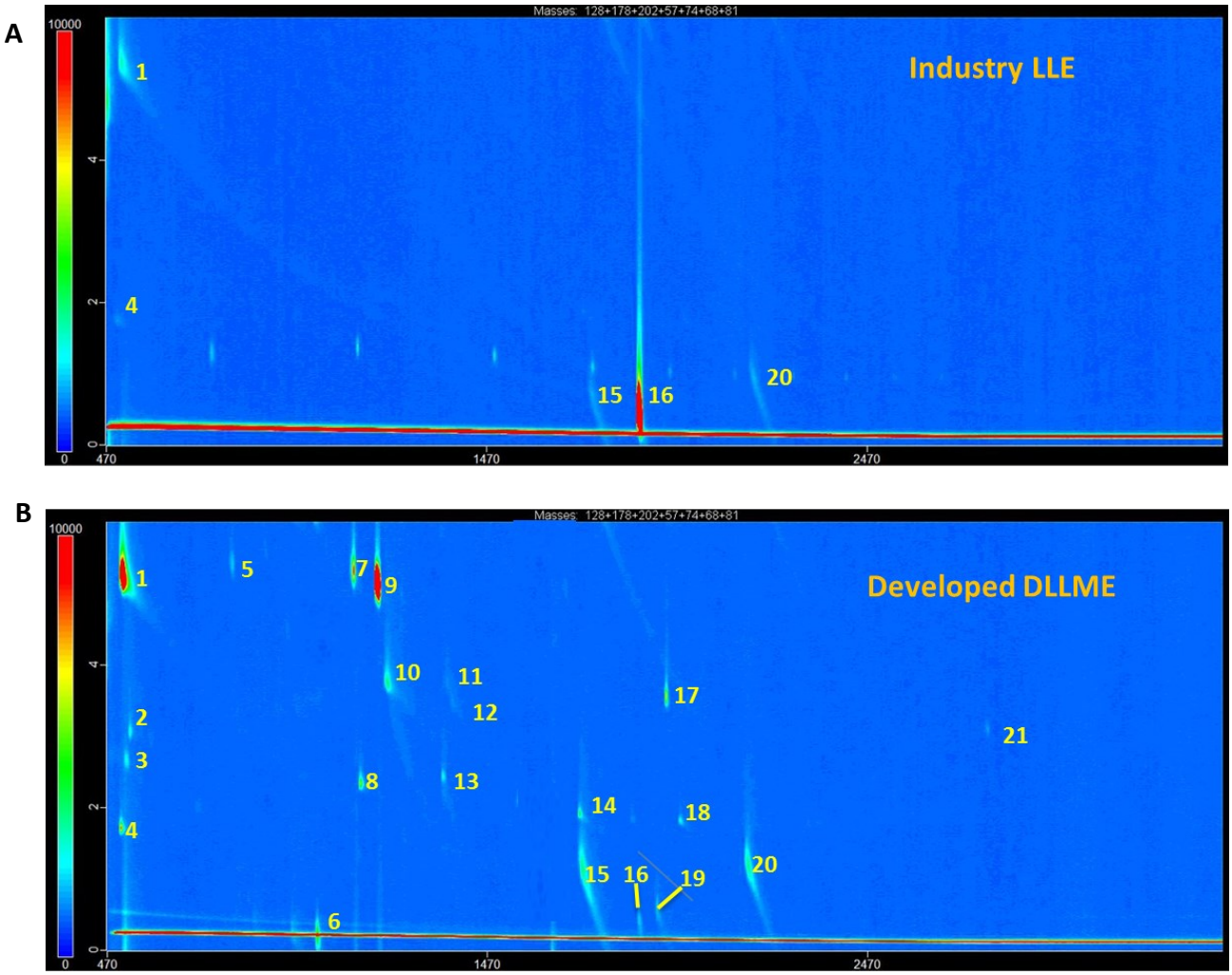


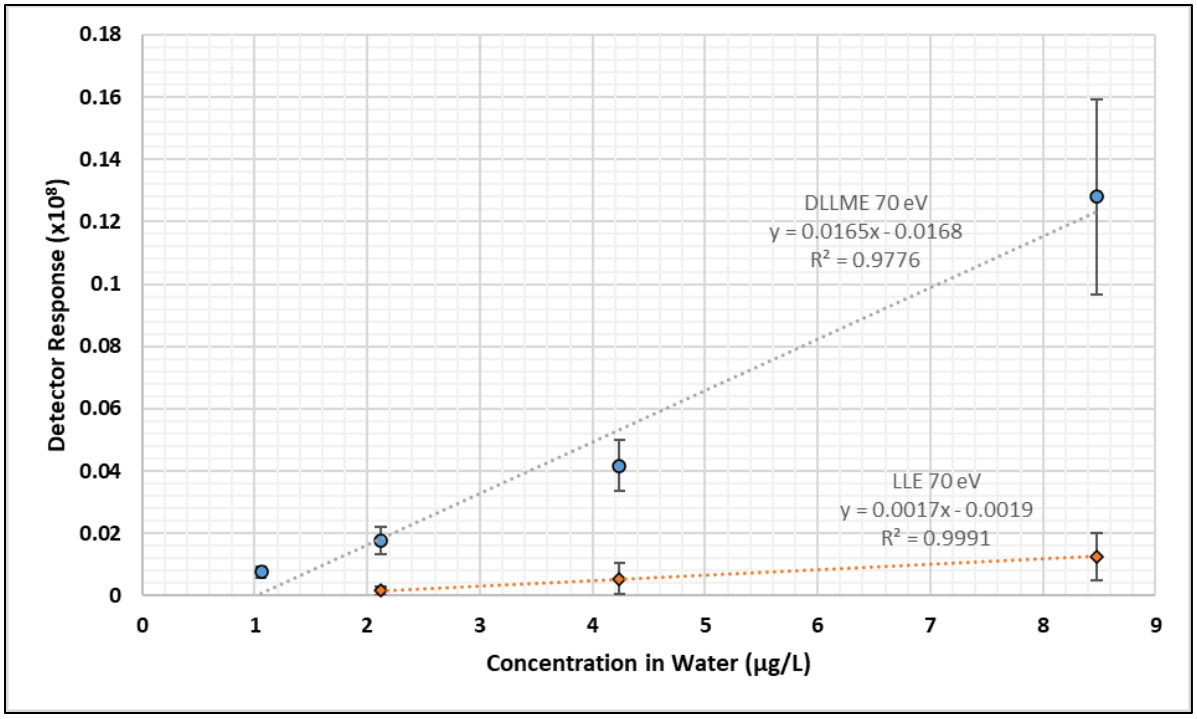
Figure 2-5. GC×GC contour plots A. LLE ; B. DLLME . Naphthalene (1), decanal (2), adamantine (3), dodecane (4), 1-naphthol (5), 2,6-dimethylnaphthalene (6), 1-naphthaldehyde (7), tetradecane (8), butylated hydroxytoluene (9), methyl laurate (10), 2-tetradecen-4-ol (11), 2-tetradecanol (12), fluorene (13), octadecane (14), phenanthrene (15), *o*-terphenyl (16), 2-heptadecanone (17), eicosane (18), 1-methylphenanthrene (19), pyrene (20), 7,12-dimethylbenz[*a*]anthracene (21).

2.3.3 Extraction and Method Characterization Using Flow GC×GC-TOFMS

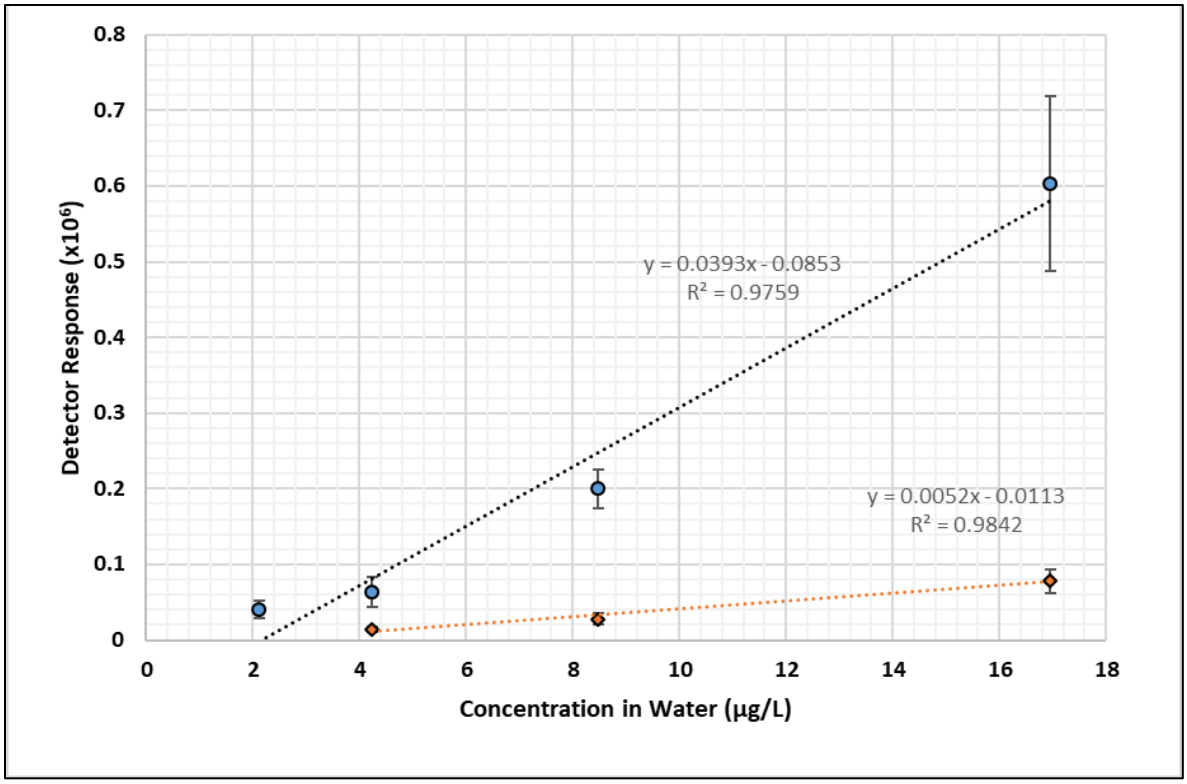
Flow-GC×GC-TOFMS/FID was used to produce calibration curves for select target compounds and for eventual characterization of real samples. Flow-GC×GC-TOFMS/FID and cryo-GC×GC-TOFMS methods had their overall chromatographic separation and oven programming set up as similarly as possible to each other to provide a continuation of the study from preliminary studies to real sample analysis. For real samples, -70 eV and -12 eV EI MS were used to identify peaks to inform the analysis by FID, which was used for quantification.

Methyl laurate is presented as an example to compare performance of the three detection channels (Figure 2-6). Figure 2-6 also shows the difference between the two extraction methods. The results show a decrease in the limit of detection (LOD) of the DLLME extraction compared to the LLE. As can be seen by the slope of the curves the DLLME method is also significantly more sensitive. Calibration curves show lower concentration standards most relevant for use with trace real sample calibration and LOD calculation. FID curves are more linear and have smaller standard deviations than the two TOFMS ionizations.

A



B



C

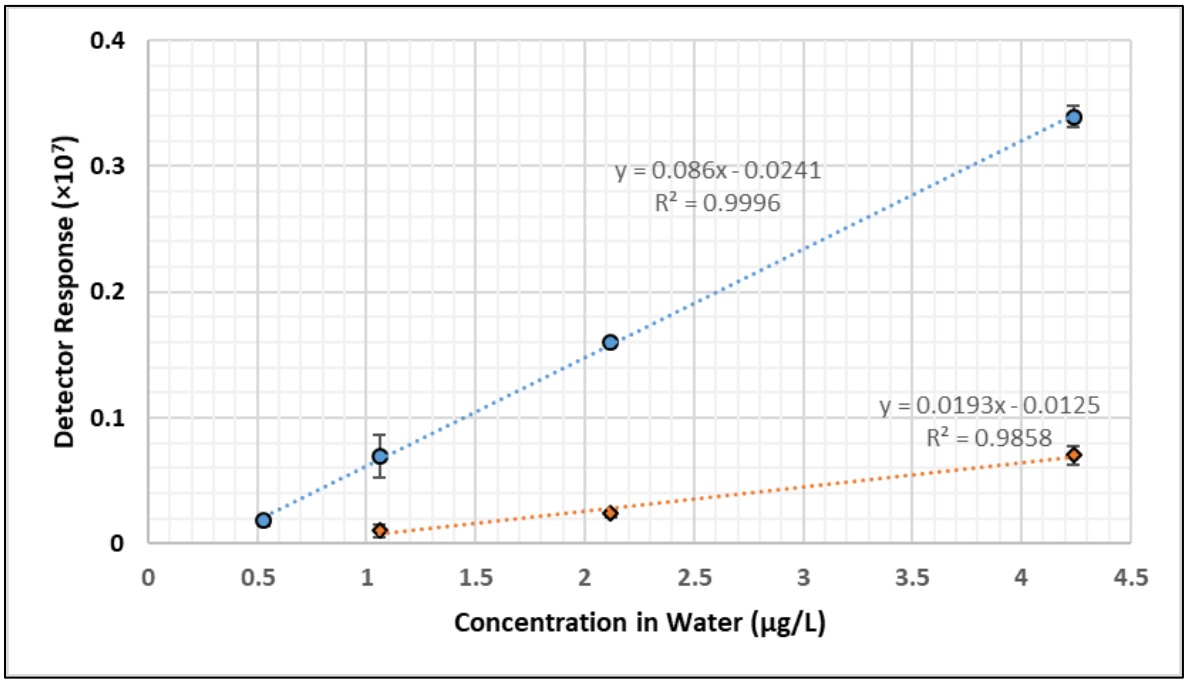


Figure 2-6. Methyl laurate calibration curve comparison between LLE and DLLME. LLE as orange diamonds, with DLLME as blue circles. Error bars represent one standard deviation (n=5). A. -70 eV; B. -12 eV; C. FID.

2.3.4 Detection Limits and Extraction Comparisons

LODs for target compounds extracted with both LLE and DLLME were compared on the basis of their responses on the cryo-based GC×GC system (Table 2-2). The DLLME method provides limits of detection that range from 17 to over 370 times lower than the LLE method.

Table 2-2. Comparison of method limits of detection (LLE vs DLLME) for target compounds using cryogenic GC×GC-TOFMS. Values are compared numerically by the ratio of the LLE LOD divided by the DLLME LOD.

Compound	LOD LLE (µg/L)	LOD DLLME (µg/L)	DLLME vs LLE Detection Ratio
naphthalene	14	0.08	1.8×10^2
phenanthrene	14	0.45	32
pyrene	11	0.27	40
dodecane	38	0.31	1.2×10^2
octadecane	78	0.25	3.1×10^2
eicosane	63	0.17	3.8×10^2
2,6-dimethylnaphthalene	12	0.36	32
1-methylphenanthrene	16	0.58	27
7,12-dimethylbenz[*a*]anthracene	27	1.6	17
1-decanal	33	0.58	56
2-heptadecanone	47	1.0	47
9'-fluoren-9-one	19	0.26	72
methyl laurate	29	0.73	40
1-naphthaldehyde	25	0.21	1.2×10^2
2-tetradecanol	29	0.49	59
2-tetradecen-4-ol	37	0.38	96
1-octadecanol	41	1.0	41

To demonstrate the potential for obtaining relative quantification concentrations for unknown compounds, a series of target compounds were spiked in water solution at a known concentration before being extracted and analyzed. The mass of each compound in this mixture was weighed, representing a gravimetric solution prep concentration. These values are represented in Table 2-3, column 5. These select compounds were quantified using a true calibration curve (i.e., a true calibration curve of octadecanol was used to quantify octadecanol). These values are represented in Table 2-3,

column 4. Finally, a “surrogate” calibration curve of a different compound with a similar response factor was used to quantify the compound (i.e., the calibration curve for octadecane was used as a surrogate compound to quantify octadecanol). These values are represented in Table 2-3, column 3. The true values are comparable to the relative quantification values calculated from column 3 in most cases. This indicates that compounds other than the compound of interest can potentially be used to obtain a relative quantification value using this method provided they have similar response factors.

Table 2-3. Comparison between the measured concentrations of “unknown” compounds quantified using a surrogate calibration curve, the true calibration curve for each compound, and the true concentration based on gravimetric solution preparation. Surrogates are compounds projected to have similar response factors to the “unknown” compound of interest.

“Unknown” compound	Surrogate calibration curve used for relative quantification	Surrogate calibration curve concentration (µg/L)	True calibration curve concentration (µg/L)	Gravimetric solution prep concentration (µg/L)
octadecanol	octadecane	2.6 ± 0.8	2.8 ± 0.4	3.0
4,4’-dichloro-1,1’-biphenyl	4-chloro-1,1'-biphenyl	2.9 ± 0.3	2.6 ± 0.2	2.3
eicosane	octadecane	5 ± 1	3.9 ± 0.3	3.6
pyrene	naphthalene	2 ± 1	2 ± 1	2.8
naphthaldehyde	methyl laurate	7 ± 1	7 ± 2	5.9
chlorooctane	decanal	3.0 ± 0.8	2.3 ± 0.4	2.8

2.3.5 Analysis of Real Samples

Figure 2-7 shows a chromatogram of a real sample contaminated with petroleum. The sample was extracted using the optimized DLLME method and analyzed using cryo-GC×GC-TOFMS. A significant amount of information about the sample can be determined, not limited to regions of TEH and TPH. Distinct regions of interest can be generated and important characteristics of the sample can easily be distinguished.

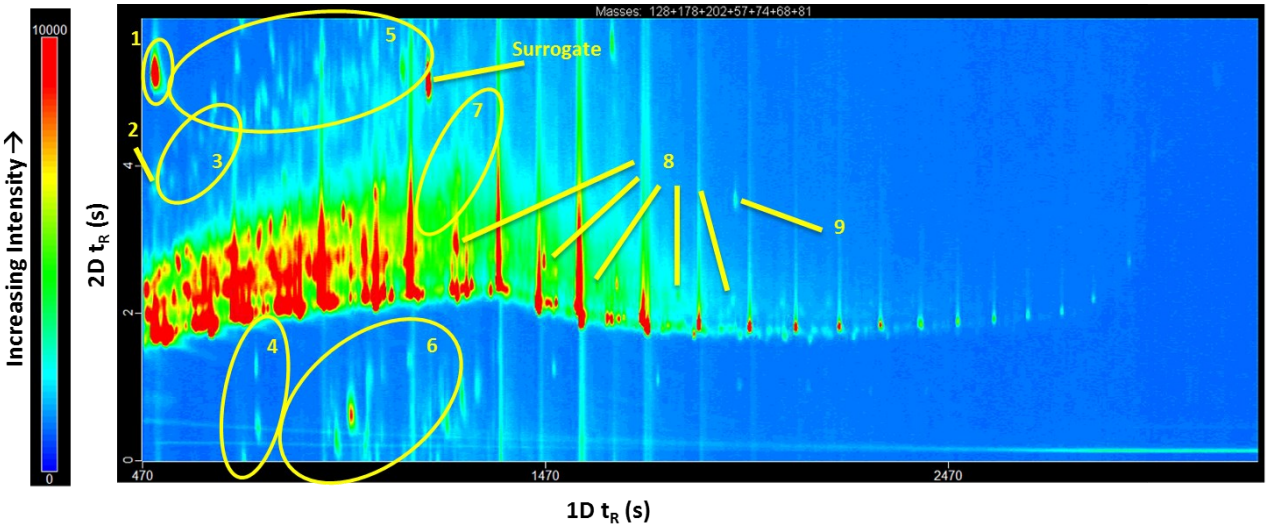


Figure 2-7. GC×GC contour plot of contaminated sample. Naphthalene (1), phenyl benzaldehyde (2), alkyl and polyalkyl benzenes (3), alkyl Indanes (4), alkyl naphthalenes (5), polyalkyl naphthalenes (6), cycloalkyl ketones and aldehydes (7), long-chain alkyl cyclohexanes (8), pyrene (9).

Since the flow-GC×GC-TOFMS/FID system was deemed more likely to be adopted by industry based on economic and maintenance factors, the 27 real samples collected from the Edmonton area were studied using the optimized DLLME protocol and analyzed using flow-GC×GC-TOFMS/FID. Chromatograms of all real samples and blanks can be found in Supplementary Information 3. Figure 2-8 presents a swarm plot of the compounds detected in each real sample. Compounds were sorted into classes for visual simplicity. Each compound identified is indicated by one circle on the figure. Samples are normalized to their respective set of surrogate and internal standard. Compounds required a library match factor > 675 to be considered as tentatively identified. Relative quantification was used to compare samples.

Samples were obtained from sampling locations and circumstances with the intention of creating variety and interesting comparisons. Samples 18 and 19, taken from a creek after a small oil spill, had significantly more contaminant compounds present at a range of concentrations. The compound class that appeared most commonly was alkanes and similar derivatives. The majority of samples were taken directly from the North Saskatchewan River, storm-fed creeks, and from urban taps (Figure 2-1). Samples 3 and 5 were taken from the point in the river where the outflow of the Goldbar Wastewater Treatment Plant mixes with the North Saskatchewan River and nearby downstream, respectively, showing significant difference between the contamination present. These two samples also showed the difference between sampling done downstream compared to upstream (Samples 2 and 4) and even 200 m further downstream (Sample 7). Unsurprisingly, the samples taken close to the outflow had

significantly more organic content in terms of both number of compounds and their concentrations than the samples that were further downstream or upstream of the treatment plant. A variety of PAHs, ketones, and polyesters were commonly present in the samples that had fewer contaminant compounds.

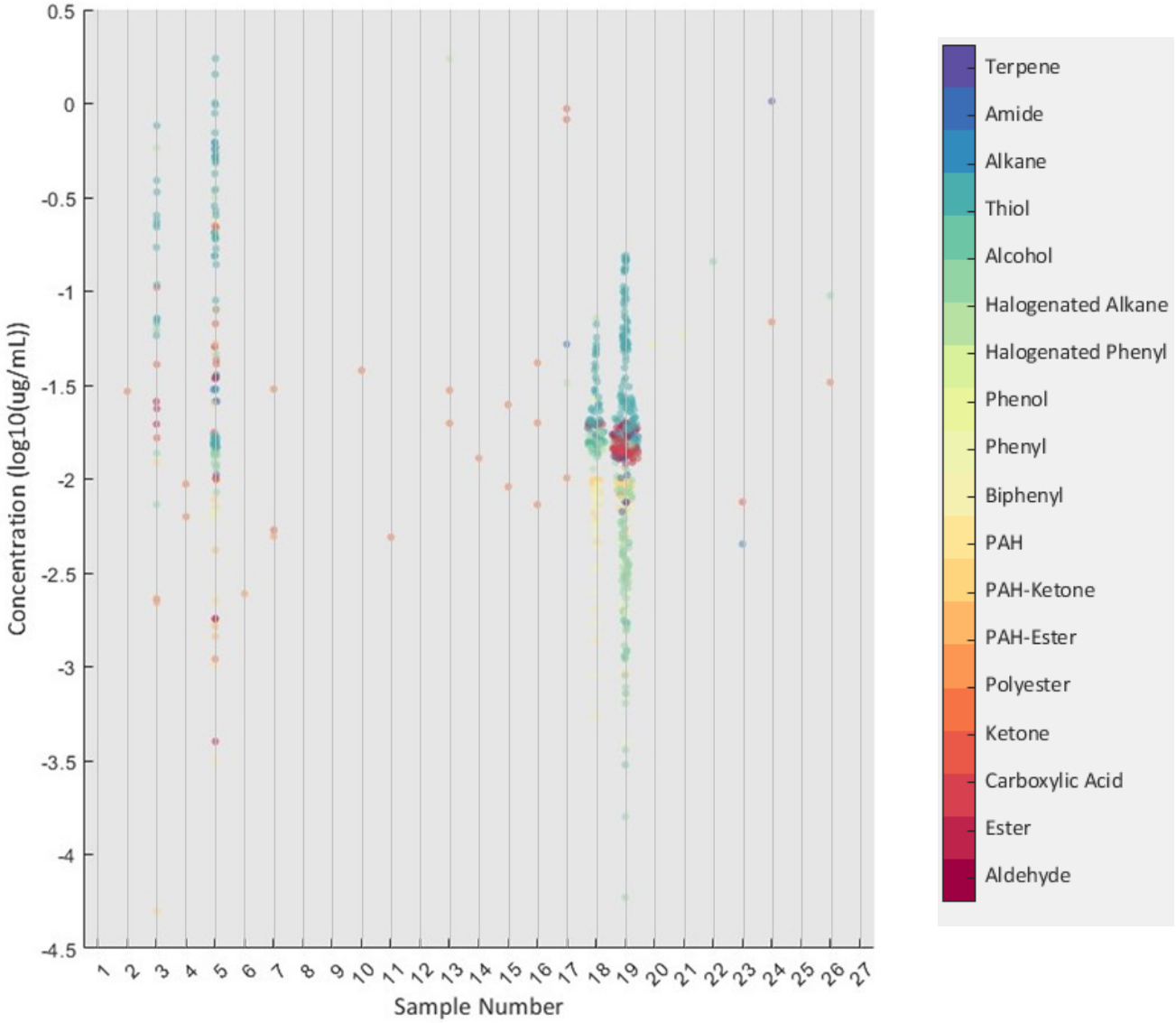


Figure 2-8. Swarm chart representing the compounds found in real samples. Each circle represents one compound. Points are distributed along the y-axis based on the log10 concentration calculated by a relative quantification surrogate. A swarm chart allows for each individual compounds to be represented visually by separating points in the x-axis when they have the same y-value.

2.3.6 Economic and Greenness Evaluation

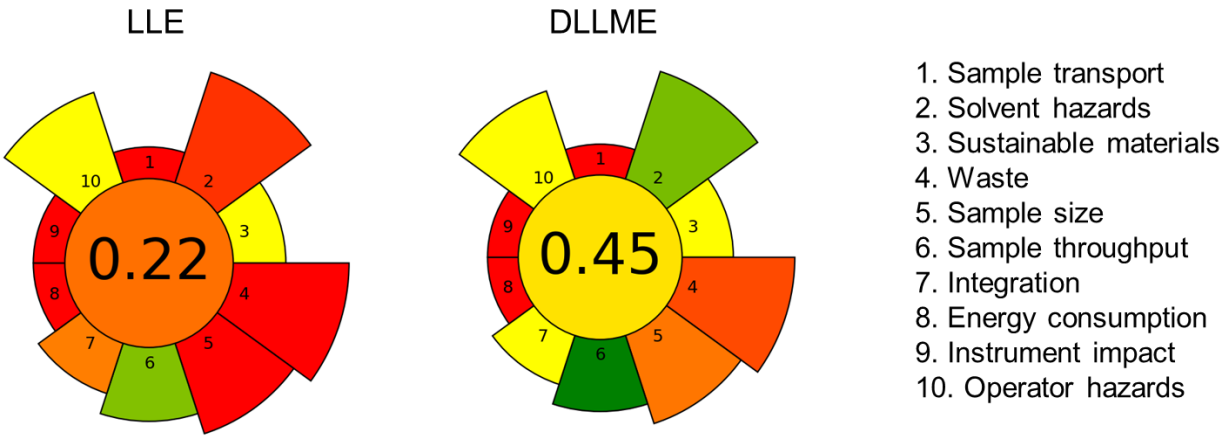
When comparing the extraction methods based on more than just their analytical merits, the DLLME method was found to be both greener and more economical for routine analytical laboratories. The greenness metric used for comparison [76] gave the DLLME method a score of 80, and the LLE method a score of 76, with scores closest to 100 being deemed more environmentally friendly. Summary of these evaluations are included in Table 2-4. Figure 2-10 uses the AGREEPREP scale introduced by Wojnowski et al, 2022 [75]. This scale includes weighted parameters and places more emphasis on waste production, solvent volume and safety for the user of the method. The AGREEPREP method more definitively shows the difference between the two methods.

Table 2-4. Analytical Eco-Scale metric designed by Galuszka et al, 2012. Indicated in the table are "penalty points" given for each of the given descriptors. The "total" is calculated by subtracting the penalty points from 100; the closer a method gets to a score of 100 the more environmentally friendly the method is considered. Appendix 1 includes the metric for evaluation by the Analytical Eco-Scale.

Metric	Hexane LLE Scoring Assignment	LLE Penalty Points	PCE DLLME Scoring Assignment	DLLME Penalty Points
Reagent Amount	<10 mL	1	<10 mL	1
Reagent Hazard	4 pictograms * danger (2)	8	2 pictograms * warning (1) 2 pictograms * danger (2)	6
Energy Use	>1.5 kWh per sample	2	>1.5 kWh per sample	2
Occupational Hazard	Emission of vapors	3	Emission of vapors	3
Waste volume	>100 mL	7	>10 mL	5
Waste treatment	No treatment	3	No treatment	3
Total		**76**		**80**

Figure 2-9. Example pictograms associated with chemical components of solvents and solvent mixtures. Shown are "Flame" (fire hazard) and "Exclamation mark" (may cause serious health effects or damage to ozone layer). The associated safety word of "Danger" or "Warning" informs the model of the intensity of the hazard.

Figure 2-10. AGREEPREP greenness comparison between the LLE and DLLME methods. Overall "greenness" is represented by the number in the middle of the wheel, with a number closer to 1 being good and a number closer to 0 being bad. Relative weighting of each of the categories is indicated by the relative size of its slice in the wheel. Colours range from green (best) to red (worst).



The cost analysis was based on real costs incurred for the materials, and estimated costs of labor and shipping. In terms of the costs of the analyses, the two methods have essentially the same cost if one ignores costs associated with transporting the samples to the laboratory. However, due to the significant decrease in sample volumes and weights, savings on sample shipment can also be realized. In our analysis we assumed standard, 5-business-day shipping from Fort McKay, Alberta to Edmonton, Alberta (a distance of 500 km) and the savings were ~50 CAD per 50 samples. Details of these calculations are provided with Table 2-5.

Table 2-5. Cost analysis comparing the two aqueous extraction methods presented.
[#]The assumption was made that the only mass accounted for would be the mass and volume of a sample of pure water that was filled to the top of each sample container.
[+]Assuming no samples are spilled, diluted, or reprepared.
*Waste disposal costs and procedures based on those used at the University of Alberta. Disposal costs for different organizations and regions may vary.

Parameter	Cost / Sample	Cost / 50 Samples
Extraction Time (1h)	↓ $0.60 ($18/h, 6/batch)	↓ $30.00
Transport using standard Canadian shipping[#]	$0.00 ($13.00)	↓ $50.00 ($117.15 -> $67.15)
Solvents used[+]	↓ $0.39 ($0.47 -> $0.08)	↓ $19.50
Purchasable consumables	↑ $0.95 ($1.15 -> $2.05)	↑ $47.50
Waste Disposal*	$0.00 (<$0.01)	$0.00
Total:	↓ $0.04	↓ $2.00 + ↓ $50.00

2.4 Conclusions

It was shown that the DLLME protocol using tetrachloroethylene as extraction solvent and acetone as dispersion solvent more efficiently extracted a wider range of analytes at lower concentrations than the conventional LLE with hexane. The method was able to perform these tasks without needing expensive automated sample extraction / preparation equipment, allowing it to be easily and inexpensively adopted in routine laboratories that may not have access to such equipment. The DLLME protocol also provided a more detailed description of the organic contents of a water sample as opposed to a conventional hexane LLE and had far greater sensitivity and a lower limit of detection. It also proved to be more cost effective and greener.

A collection of compounds was able to be detected and analyzed to determine their approximate concentration using calibration curves of related compounds. Based on these findings this semi-

quantitative approach is certainly viable for larger scale studies for hybrid target/non-target analysis. As for real samples taken from the environment, a wide range of compounds and compound classes were able to be analyzed using DLLME-GC×GC-TOFMS/FID as a non-targeted approach. This approach can then be used to quickly screen samples for unexpected compounds, while also providing semi-quantitative concentrations, clearly indicating which compounds may be present in a sample at levels that may be of concern. Since it is impractical to develop and maintain calibrations for all possible compounds that may be encountered in a water sample, this type of information is invaluable for quickly indicating those compounds found in a sample that would be worthy of the effort and expense of generating an individual calibration curve for more accurate targeted analysis.

Chapter 3: Comparison of Extraction Solvents and Thermal Desorption for the Analysis of Solid Environmental Matrices Using GC×GC-TOFMS

3.1 Introduction

The Alberta economy is heavily tied to large-scale projects extracting natural resources from the earth. The most high-profile of these is Alberta's oil sands, which receive over $20 billion (CAD) investment annually and make up over 20% of the province's gross domestic product [101,102]. The oil sands also regularly make headlines as a significant source of environmental soil contamination [103-105]. Soil analysis in industry, therefore, is often done to determine the presence and extent of contamination from petroleum products. Among the lesser known of the province's resources are Alberta's shale deposits. Since shale fracturing and extraction is a part of the Alberta oil and gas industry, the industrial analysis of shale is often done not with the intention of determining contamination sources, but rather to determine the composition of the shale rock and the organic compounds contained within [106,107].

As with water analysis, the methods used in solid matrix environmental analysis are relatively outdated, with many methods in current use dating back more than two decades [108,109]. Methods for the analysis of soil, and especially shale tend to target lists of compounds that are the most basic components of petroleum, such as linear and branched alkanes [109,110]. These methods explicitly consider the of aromatics, cycloalkanes, and other common petroleum components present in a sample; however, they also miss out on many compounds and compound classes that could make a shale deposit or soil contamination site unique.

One thing that is not clear whether modern contract labs are doing is statistically analyzing analytical methods against each other and testing small changes that could improve their methods. One isolated alteration that can be made without needing to make large changes in infrastructure is the method of extraction and sample preparation. The use of organic solvents is by far the most common method of extraction used for gas chromatography systems. The gold standard of these is Soxhlet extraction, but simpler and much faster methods designed to agitate solid matrices with solvents (also known as "cold shake" methods) far exceed the use of Soxhlet in industry largely based on economic factors [109,111]. Evaluation of how effective cold shake methods are compared to Soxhlet is important to understand what industrial methods could be missing out on by opting for faster, less expensive extraction methods.

Many labs have protocols that use a range of extraction solvents, with hexane, hexane/acetone mixtures, dichloromethane, toluene, methyl-*tert*-butyl ether, and ethyl acetate being among the most common [111,112]. Thermal desorption is also becoming increasingly popular, but a high upfront cost and specific equipment/infrastructure requirements often make labs hesitant to adopt its use [109]. Evaluating which of these extraction systems is most effective for different compound classes and overall non-targeted analyses could hopefully pave the way to the development of standardized metrics that would be able to recommend a solvent and extraction type for an analysis being performed.

There are several components that can come together to make a successful evaluation of sample introduction methods. Sophisticated separation instrumentation such as GC×GC is optimal to gain information about the compounds present in a sample. TOFMS with multiple ionization energies allows for the tentative identification of compounds, the comparison of response intensities within samples, and ultimately evaluation of sample introduction methods used to analyze a consistent sample type. Scripts embedded in processing software can help to easily classify compounds to aid in evaluation. Other metrics based around factors such as monetary cost, environmental impact and ease of use would also be of interest to the environmental analysis industry. Ease of use was not evaluated in this study but was made a consideration for all steps of analysis. The novel use of scripts to classify and sum compounds into distinct classes was the main focus of this study. This allowed the decision of which solvent was most effective for a given type of analyte to be made with much more efficiency than what is currently done in industry.

3.2 Materials and Methods

3.2.1 Sample Preparation

3.2.1.1 Reagents and Solvents

Extraction solvents used for liquid extractions included HPLC grade acetone, HPLC grade ethyl acetate, ACS toluene, ACS *tert*-butanol (all Fisher Scientific, Ottawa, ON), ACS dichloromethane, HPLC grade tert-butyl methyl ether (Millipore-Sigma Co., St. Louis, MO) and environmental grade n-hexane (Alfa Aesar, Haverhill, MO). Internal standards used included n-nonane-d20 (CDN Isotopes, Pointe-Claire, QC) and *o*-terphenyl (K&K Laboratories Inc., CA). Napthalene-d8 (Isotec Inc., Miamisburg, OH) was used as a surrogate. Consumables used for final preparation included glass 2mL GC vials with PTFE screw caps (Chromatographic Specialties Inc., Brockville, ON). Glass wool was 8-micron fiberglass (Corning Inc.

Corning, NY) and inert sand was sea washed sand (Fisher Scientific, Ottawa, ON). Glass wool and inert sand were both baked at 400°C for a minimum of 5h before use in analyses.

3.2.1.2 Soil

Six soil samples were collected in 100 mL screw-capped glass jars from various locations around Hastings Lake, AB. The samples were homogenized and divided into smaller portions, frozen at -7 °C, then ground and homogenized using a mortar and pestle (CoorsTek Inc., Lakewood, CO) over dry ice and shaken through a 300 µm mesh hop sieve (LuckyHigh, Naypyidaw, Myanmar). The homogenized samples were then mixed in varying amounts to form 15 working soil samples (Soil1-Soil15). Water content of samples for determination of dry weight was measured by baking samples in an industrial oven at 60 ^{o}C for 4 h. Soil1-Soil15 were stored in scintillation vials at 4 °C until being allowed to come to room temperature for extraction.

3.2.1.3 Shale

All shale used in this study was taken from shale core samples provided by an industrial collaborator (AGAT Laboratories , Calgary, AB). 7 shale samples (Sh1-Sh7) were formed by taking shards of shale core, grinding them using a mortar and pestle, and shaking them through a 300 µm mesh hop filter. Sh1-Sh7 were also stored in scintillation vials at 4 °C. All shale samples were spiked with a surrogate, Napthalene-d_8 in acetone at an effective 10 µg/g. Water content of shale samples was initially measured and determined to be negligibly low; water content values were subsequently not recorded for shale analyses.

3.2.2 Liquid Extractions

Figure 3-1 shows a schematic of the liquid extraction procedure used, starting with a homogenized solid sample. 0.2 g of soil or shale was added to a 1.5 mL safe-lock, PCR clean centrifuge tube (Eppendorf Canada, Mississauga, ON), followed by the addition of 1 mL of solvent using a 100-1000 µL positive displacement pipette (Mandel Scientific Company Inc., Guelph, ON). This was then shaken for 5 minutes at 1000 rpm using a BenchMixer™ V2 vortex mixer with a multi-tube attachment (Benchmark Scientific, Sayreville, NJ) and then centrifuged at 2000 rpm for 10 minutes using a MIKRO 185 micro centrifuge (Hettich Instruments LP, Beverly, MA). The supernatant was transferred to a GC vial after being filtered by gravity filtration using glass wool packed into a glass Pasteur pipette. A 450 µL aliquot was taken with a 100-1000 µL positive displacement pipette and added to a vial containing 50 µL of internal standard in acetone, consisting of *o*-terphenyl and n-nonane-d_{20}, in respective concentrations of 1.4 and 1.1 µg/mL.

The cold shake liquid extraction process was repeated for Soil1-Soil15 and done in triplicate for Sh1-Sh7 for each solvent system.

Figure 3-1. Extraction schematic for the preparation of homogenized soil and shale using cold shake liquid extraction. Image produced using BioRender™ figure generation software.

Soxhlet extractions were compared to cold shake extractions using two solvents, hexane and ethyl acetate according to a modified version of EPA method 3540C [113]. 4 g (± 0.4 g) of sample was weighed on an analytical balance with 4 g (± 0.4 g) of anhydrous sodium sulfate as a drying agent. The two solids were shaken thoroughly in a 20 mL glass scintillation vial before being added to a Soxhlet extractor between two layers of oven-baked glass wool. A 100 mL round bottom flask with 80 mL of solvent and pre-spiked internal standard was attached to the bottom of the extractor with a fractional distillation column attached to the top. Internal standard volume was adjusted to reflect the 1.4 and 1.1 µg/mL of *o*-terphenyl and n-nonane-d20 by increasing the volume of spiked standard 80×. Temperatures were set using heating mantles so that each solvent underwent 5 cycles through the extractor per hour. The Soxhlet was left for 20 hours before removing the heating mantle and collecting the solvent in an adaptor vessel for a Rocket Synergy 2 Evaporation System (Thermo Fisher Scientific, Waltham, MA). The sample was dried down according to the manufacturer's specifications and reconstituted in 1 mL of solvent before analysis using GC×GC-TOFMS. Alternatively, a Kuderna-Danish apparatus in a hot water bath could also be used for this dry-down step.

3.2.3 Thermal Desorption Extractions

Glass TD tubes (Supelco Inc., Fisher Scientific, Ottawa, ON) were prepared as described by Piotrowski et al, 2018 [114]. In brief, tubes were packed with glass wool, sand, then 10-16 mg of soil or shale, followed by another layer each of sand and glass wool to contain the sample within the glass tube. The tube was then loaded in a TD-100xr thermal desorption unit (SepSolve Analytical, Waterloo, ON).

3.2.4 GC×GC-TOFMS Analysis

A 7683 series auto-injector (Agilent Technologies, Santa Clara, CA, USA) was used to inject liquid samples (1 µL) into a split-splitless injector used in splitless mode and set to 300 °C. For TD samples a TD100-xr thermal desorption unit (Markes International Ltd., Waterloo, ON) was used for sample introduction. Samples were desorbed for 15.0 min at 250 °C with a trap flow of 50 mL/min nitrogen. Trap settings were 1.0 min trap pre-purge at 20 mL/min and setting the trap temperature to -25 °C. Desorption of the trap involved rapidly heating to 300 °C for 5 min with a split of 70 mL/min with 0.73 mL/min on column. The transfer line to the GC oven was maintained at 150 ^{o}C.

Analyses were performed using an Agilent 7890A chromatograph (Agilent Technologies, Santa Clara, CA) fitted with an INSIGHT flow modulator operated in reverse fill-flush mode (SepSolve Analytical, Waterloo, ON). The first dimension column was a 30.0 m × 0.25 mm; 0.25 µm film thickness Restek Rtx-5 (Chromatographic Specialties, Brockville, ON, Canada). The second dimension column was a 5.0 m × 0.25 mm Rtx-200 phase with a thicknesses of 0.25 µm (Chromatographic Specialties, Brockville, ON, Canada). 5.0 grade helium (Linde Gas & Equipment Inc., Burr Ridge, IL, USA) was used as the carrier gas at 0.73 mL/min in the first dimension and 15.4 mL/min in the second dimension. After the second dimension column a two-way purged splitter was used to divide flow between an FID and mass spectrometer. The modulation period was set to 2.3 s (flush 230 ms). A 2.8 m × 0.1 mm deactivated fused silica bleed line was used for the modulator. The oven program was 40 °C (4 min hold), 5 °C/min to 300 °C (10 min hold). The FID was operated at 300 °C with flows of 40 mL/min H_2, 250 mL/min air, and 20 mL/min makeup gas (He), respectively. The mass spectrometer was a BenchTOF-Select mass spectrometer (Markes International Ltd, Bridgend, UK), operated in tandem electron ionization mode (-16 eV and -70 eV) at 100 spectra/s in each mode. Filament voltage was set to 1.5 V, and a mass range of 40-600 m/z was selected.

3.2.5 ChromSpace Processing of Data

Data was processed using ChromSpace® version 2.0.2 (Markes International Ltd., Llantrisant, UK). Three different processing methods were used, one for each of the three methods of detection (-70 eV, -16 eV, and FID). The -70 eV electron impact (EI) TOFMS processing method utilized a curve fitting algorithm with 5 pseudo-Gaussian smoothing points. A minimum peak area was set at a value of 10000 counts^2, with a minimum height of 10000 counts and width of 0.050 minutes. NISTMS search 2.0 and Wiley 2008 databases were used to compare with mass spectral data obtained from individual peaks. A match of 675/1000 was considered the minimum tentative match for the identity of compounds. These matches were used as points of comparison, but were not used in the assignment of compound classes. The soft ionization -16 eV EI TOFMS processing method also utilized a curve fitting algorithm with 5 pseudo-Gaussian smoothing points. A minimum area of 5000 counts^2 and minimum height of 5000 counts was considered for the sizes of peaks. The width parameter was set at 0.050 minutes.

Scripts were made using common fragmentation patterns of compound classes found in processed data. Scripts design was based on the work for GC×GC-TOFMS metabolomics data described by Nam *et al*. (2021) [115]. The information obtained from scripts was used to label chromatographic peaks in the samples run, which were then grouped into compound class categories. The peak areas in these categories were then summed to give final values for extraction comparisons using an in house Matlab® (R2022a) script [116].

3.3 Results and Discussion

3.3.1 Soil Characterization

Figure 3-2 shows side by side total ion chromatograms (TICs) of an ethyl acetate extraction using each of the Soxhlet and cold shake methods. Though not corrected for mass, this comparison clearly shows Soxhlet extraction has a more efficient extraction than the cold shake method. When corrected for mass, Soxhlet extraction peaks still have a higher intensity and account for slightly higher recovery compared to the cold shake method. The cold shake method also tended to have fewer peaks present than the Soxhlet method throughout the chromatogram. Since cold shake extraction is more common in industry and significant time, solvent and waste disposal resources could be saved by using the cold shake method, it was used for the rest of the study. However, potentially useful information that a cold shake method misses out on could be accounted for by using a more exhaustive extraction method such as Soxhlet.

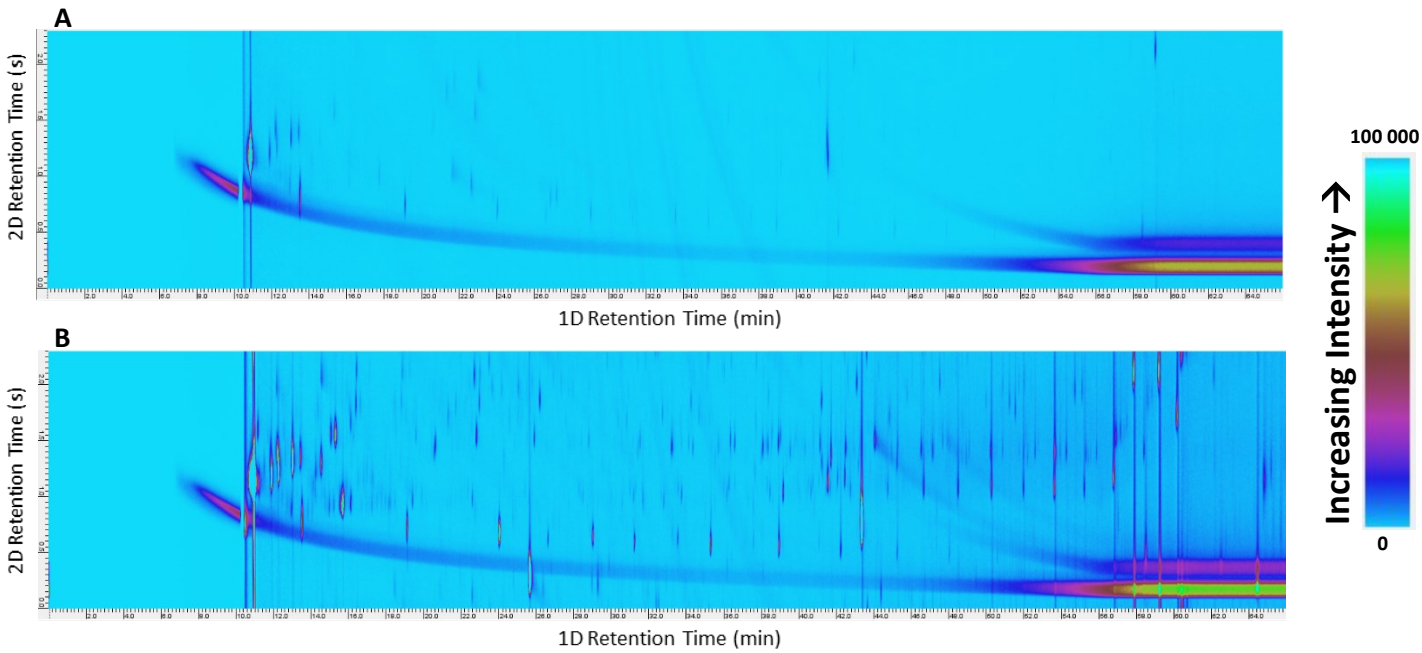
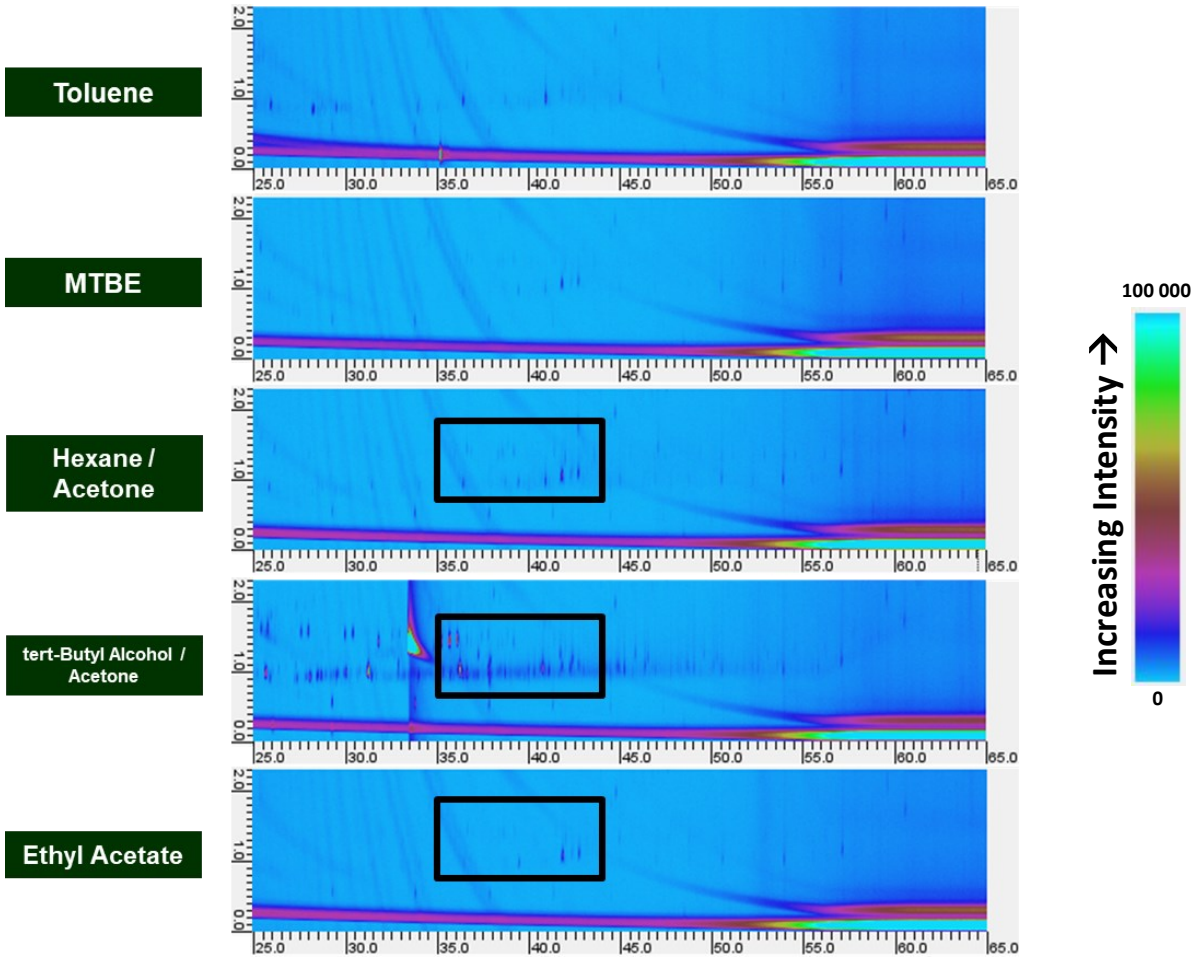


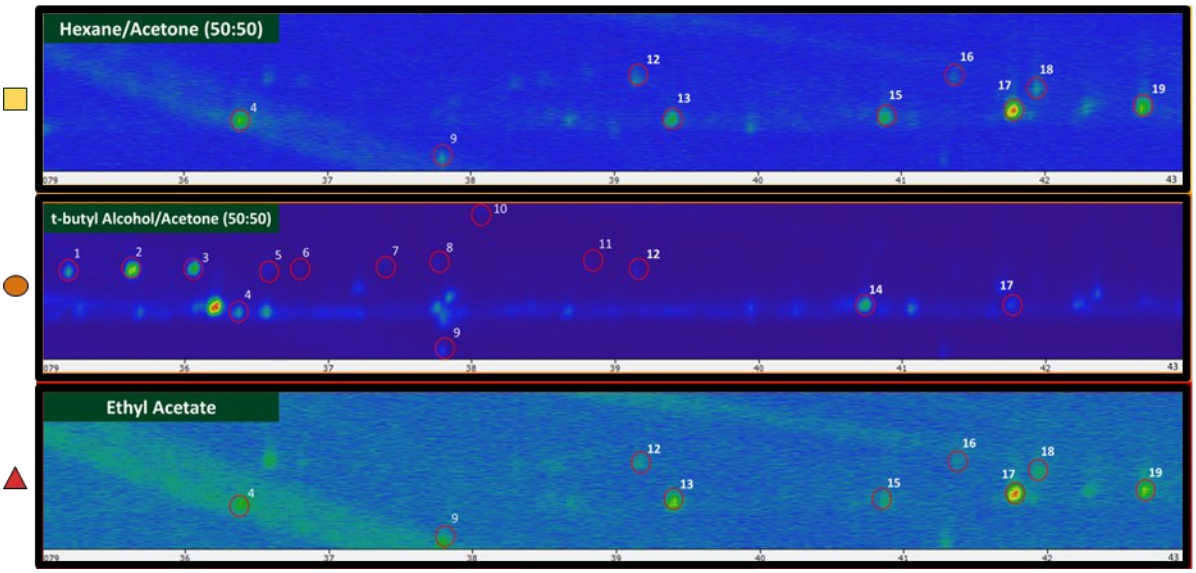
Figure 3-2. Identical samples extracted using the described (A) cold shake and (B) Soxhlet extraction methods.

Soil samples were used as exploratory tools to determine qualitative trends between extraction methods using real samples. Peak intensities and comparisons between classes of compounds were made to help inform analyses done using shale samples. Figure 3-3A shows overall TICs of the liquid extractions for a selected soil sample. Figure 3-3B includes the highlighted sections of Figure 3-3A and outlines some peaks of interest between the extractions.

A


Toluene
MTBE
Hexane / Acetone
tert-Butyl Alcohol / Acetone
Ethyl Acetate
100 000
Increasing Intensity
0

B


Hexane/Acetone (50:50)
t-butyl Alcohol/Acetone (50:50)
Ethyl Acetate

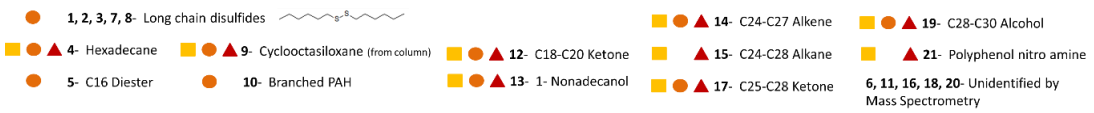


Figure 3-3. Selected chromatogram sections and selected peaks of interest for an identical homogenized soil sample. (A) Overall TIC of each given extraction. (B) Selected sections of the chromatograms in A with compounds of interest highlighted and labeled. Peaks not labeled are peaks that were also present in solvent blanks.

It was clear from initial results that there were a significant number of background peaks associated with *tert*-butyl alcohol/acetone extractions. However, this same solvent tended to be exceptional at extracting sulfur-containing compounds, nitrogen-containing compounds and alcohols compared to other solvents. Ethyl acetate tended to have a smaller signal to noise ratio than other solvents, though it was relatively comprehensive in terms of the number of compounds and classes that it was able to extract. Hexane/acetone extractions (the most common extraction for soil extractions in modern industry) was not quite as comprehensive as ethyl acetate, but had a higher signal to noise ratio. Hexane alone as a solvent was more selective for linear alkanes in the samples. Toluene was effective for the extraction of aromatics, naphthalenes and other PAH compounds, but had low overall signal for all compounds extracted. Methyl *tert*-butyl ether (MTBE) had a low signal to noise ratio and a poor coverage of compounds and compound classes. In addition to MTBE being one of the most expensive and least environmentally friendly of the solvents used during the study, these factors made it the least effective solvent of the samples tested. MTBE as a result was not considered as a solvent to continue to the analysis of shale samples.

3.3.2 Shale Characterization

With the preliminary information gained from soil characterization, the analysis of shale was done with real samples received from an industry collaborator. Scripting was added through the settings of processing methods to add compound class information to identified peaks. The use of two different ionization energies (soft -16 eV and hard -70 eV) benefited the analysis by allowing for multiple avenues of classification depending on which ionization energy was used. Phenyl-containing compounds were best analyzed using -70 eV ionization, while alcohols and cycloalkanes were most effective using -16 eV. Table 3-1 is a summarization of this information for each indicated class. Azulenes were not found at all in 5 out of 7 samples analyzed, so were not used in later comparisons for simplicity. Consistent class analyses of nitrogen-containing compounds, sulfur-containing compounds and ketones were not able to be made, so these were left out of final comparisons.

Table 3-1. Ionization energies most effective for the classification of indicated compound classes.

Analyzed with -70 eV ionization	Analyzed with -16 eV ionization
aromatics	
indanes	linear alcohols
naphthalenes	non-linear alcohols
azulenes	cycloalkanes
biphenyls	
PAHs	

Blank characterization of each sample was also of importance when considering the effectiveness of each extraction. Figure 3-4 compares blank runs of each solvent extraction consisting of the solvent and the extraction tube, and a TD system blank consisting of inert sand and pre-cleaned glass wool. Internal standard is indicated in each of the solvents by "IS". Peaks found within blanks were consistent between replicate blank runs, so blank subtraction was utilized for the analysis of real samples to prevent false positives from skewing the results observed. Of particular concern were the number of peaks present in *t*-butyl alcohol and early in the chromatograms of each of the other solvents. TD was especially clean by comparison, making it favourable for avoiding any potential false positives coming from the solvent or extraction process.

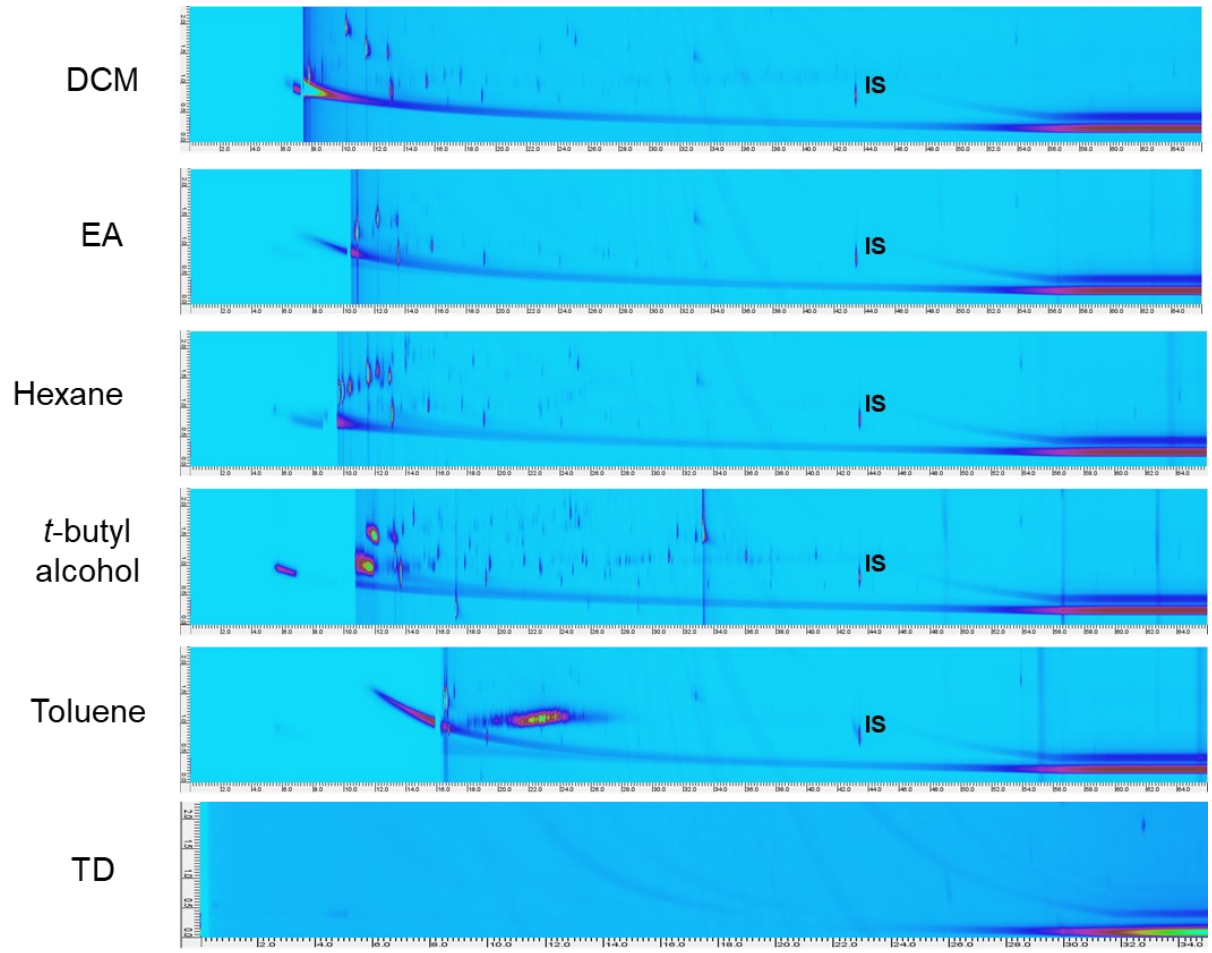
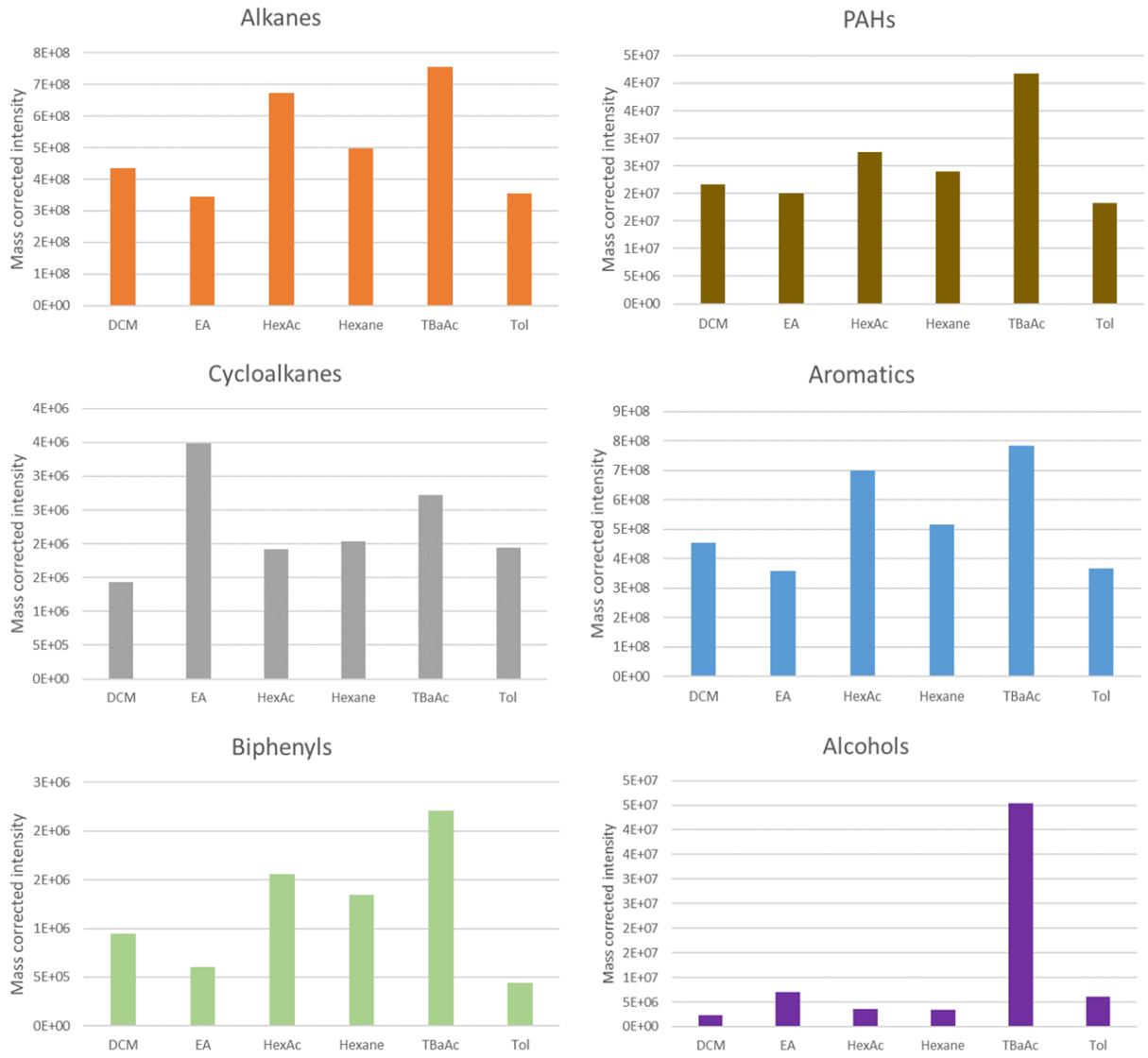


Figure 3-4. Solvent blanks (with internal standard, "IS") and a TD system blank. Peaks observed were accounted for and subtracted from summed values used in quantification of their respective compound classes.

Figures 3-5A and B show comparisons of compound classes analyzed. A total of 7 shale samples were analyzed by each extraction system to produce the data, which are displayed as sums of the combined samples. Figure 3-5B quite definitively shows the extraction capability of thermal desorption compared to any of the liquid extractions when corrected for mass. Observation of Figure 3-5A indicates that the 50:50 mixture of *tert*-butyl alcohol and acetone produced the most intense overall peak areas for all aromatic containing compounds analyzed, with 50:50 hexane/acetone consistently the next highest intensity. Being an alcohol capable of hydrogen bonding, *t*-butyl alcohol would be expected as the most effective at extracting alcohols. For alcohol containing compounds the *tert*-butyl alcohol / acetone mixture was exceptionally effective. Of the single solvent solutions, dichloromethane and hexane were the most effective, with toluene and ethyl acetate having the lowest overall intensities.

A


Alkanes
Mass corrected intensity
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol
PAHs
Mass corrected intensity
5E+07
4E+07
4E+07
3E+07
3E+07
2E+07
2E+07
1E+07
5E+06
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol
Cycloalkanes
Mass corrected intensity
4E+06
4E+06
3E+06
3E+06
2E+06
2E+06
1E+06
5E+05
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol
Aromatics
Mass corrected intensity
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol
Biphenyls
Mass corrected intensity
3E+06
2E+06
2E+06
1E+06
5E+05
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol
Alcohols
Mass corrected intensity
5E+07
5E+07
4E+07
4E+07
3E+07
3E+07
2E+07
2E+07
1E+07
5E+06
0E+00
DCM
EA
HexAc
Hexane
TBaAc
Tol

B

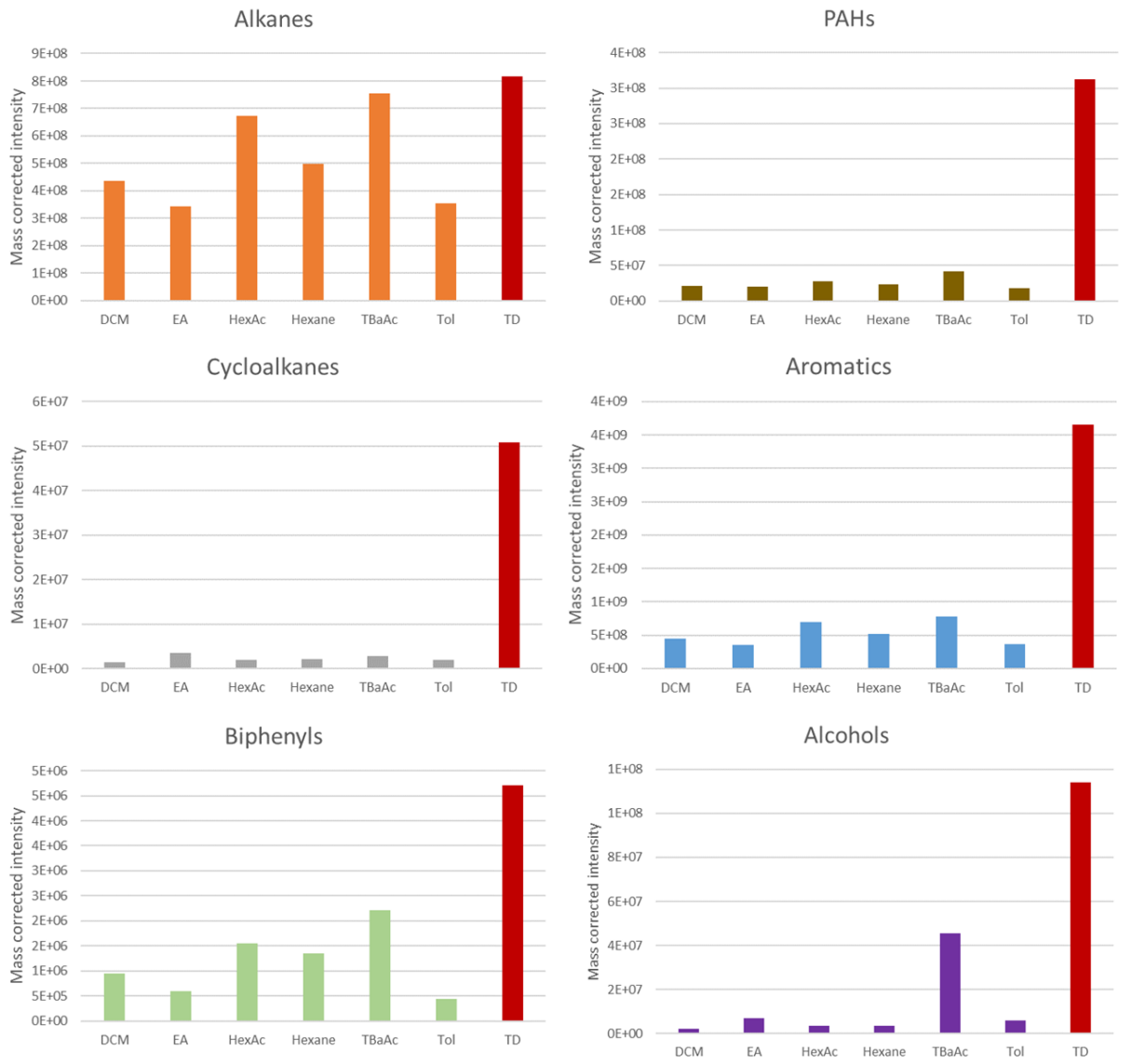


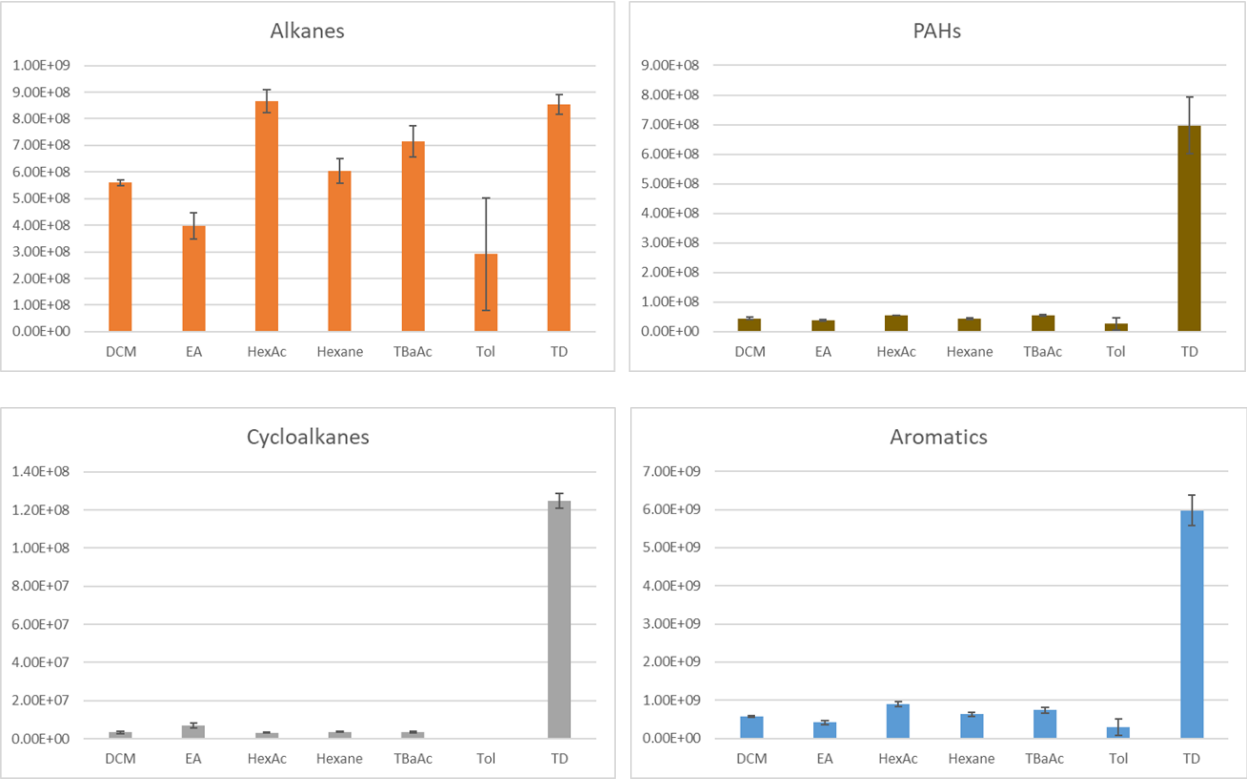
Figure 3-5. (A) Compound class sums for classes of interest. Comparisons made between solvents only (B) Compound class sums for classes of interest. Comparisons made include TD extractions and solvent extractions. TD extraction sums shown in red.

Ethyl acetate appears to have a slight edge in extraction of cycloalkanes, with DCM, hexane, hexane/acetone and toluene very comparable to each other. Ethyl acetate is also the most effective for extracting alcohols aside from *t*-butyl alcohol / acetone. Ethyl acetate's ester allows it to form hydrogen bond interactions, similar to *t*-butyl alcohol, which increases its effectiveness in extracting alcohols and other hydrogen bonding functional groups. DCM, toluene and the two solvent systems containing hexane would be expected to be relatively effective at extracting non-polar cycloalkanes but performed

slightly worse than ethyl acetate in that regard. It is unclear exactly why this is the case since all parameters used were equivalent and extraction time was deemed to be sufficient for all solvents.

Regardless of the compound being considered, thermal desorption consistently extracted the most of each compound class of interest when corrected for sample mass. Additionally, thermal desorption does not rely on the chemistry of a solvent to perform an extraction, but the volatility of the compounds in a sample. This makes it a clear choice (if available) for non-targeted analysis of volatile samples and for reducing the amount of sample needed for analysis. Thermal desorption was required to be run with a split and with a very small amount of sample, mixed with inert sand. These factors could impact the detection of low intensity peaks that would be lost in a split run.

Individual sample repeatability was also run for each of the extraction types. An example is shown in Figure 3-6 where all three replicates of Sample 7 were averaged and given a standard deviation, making up the error bars shown. Smaller standard deviation indicates superior repeatability of a given extraction. TD had a comparable or superior repeatability to all liquid extractions. Toluene seemed to vary wildly in its extraction ability between samples, meaning it would not be a good choice for this sort of extraction with a short agitation time. All other extractions seemed to have fairly stable relative standard deviations (RSD) consistently less than 10%. Toluene had an RSD consistently above 50%.



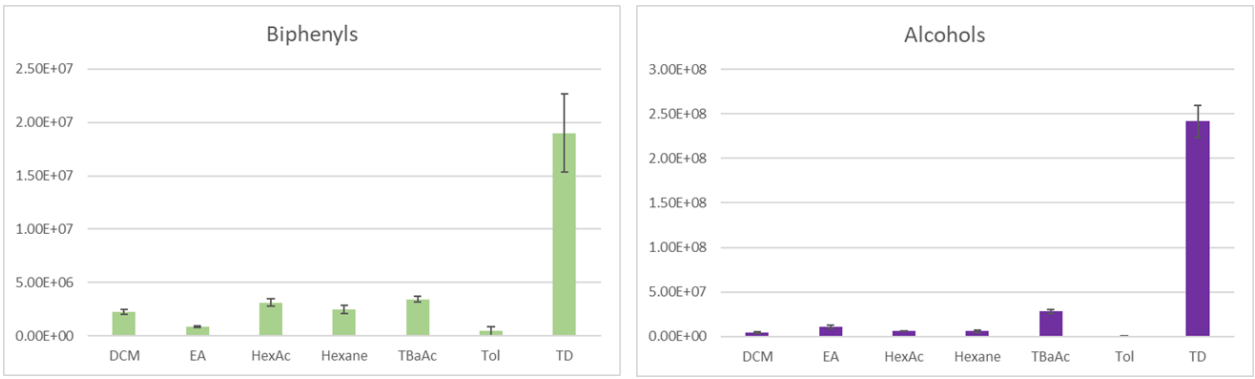


Figure 3-6. Sample 7 repeatability as shown by standard deviation of triplicate sample runs. Error bars are represented by one standard deviation (n=3).

3.3.3 Greenness and Economic Evaluations

Figure 3-7 shows the notable difference between the solvent extractions done and TD in terms of their greenness. Unfortunately, AGREEPREP focuses more on the volume of solvent rather than its hazard, so comparisons between solvents showed no differences. Table 3-2 summarizes the differences between each extraction method using the analytical eco-scale to evaluate the greenness of each method of extraction. Higher totals (fewer penalty points) indicate greener methods. It should be noted that since all liquid extractions had the same procedure, volume, and sample mass, their only figure of merit to evaluate against each other is the hazards associated with the solvent. Thermal desorption, of course, did not use solvents, allowing it to pose no reagent hazards or occupational hazards associated with the fumes released and physical exposure to chemicals.

Figure 3-7. AGREEPREP scale comparison between liquid extraction and thermal desorption of soil/shale samples.

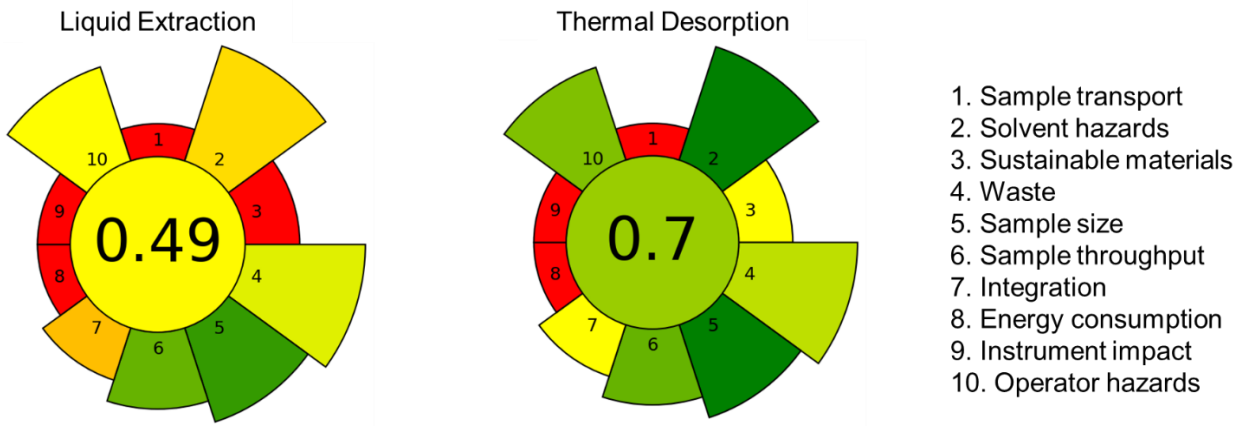


Table 3-2. Analytical Eco-Scale metric designed by Galuszka et al, 2012 [117]. Indicated in the table are "penalty points" given for each of the given descriptors is column 1. All SDS information was acquired from www.sigmaaldrich.com.

Metric	Dichloro-methane	Ethyl Acetate	Hexane: Acetone	Hexane	t-Butyl Alcohol	Toluene	MTBE	Thermal Desorption
Reagent Amount	1	1	1	1	1	1	1	0
Reagent Hazard	2	4	12	8	8	6	8	0
Energy Use	2	2	2	2	2	2	2	2
Occupational Hazard	3	3	3	3	3	3	3	1
Waste amount	3	3	3	3	3	3	3	3
Waste treatment	3	3	3	3	3	3	3	3
Total (100 – penalty)	**86**	**84**	**76**	**80**	**80**	**82**	**80**	**91**

Using this particular metric, thermal desorption is well ahead of the solvent extractions. When considering the solvent extractions, dichloromethane was deemed the greenest of the solvents included, with hexane, MTBE and the two-solvent systems of t-butyl alcohol/acetone and hexane/acetone being the least green. This metric does, however, favour dichloromethane due to its low number of reagent hazard pictograms listed in its safety data sheet. Chlorinated solvents are widely considered to be toxic, with dichloromethane itself listed as a priority substance in the Canadian Environmental Protection Act's List of Toxic Substances [108]. Ethyl acetate and t-butyl alcohol are not explicitly named in the list, but there are closely related compounds (structurally) that are present in it.

As mentioned previously, the addition of a thermal desorption unit would entail a high upfront cost, often starting at over 40 000 CAD for the least expensive units. Also associated with the addition of a thermal desorption unit would be the costs of carrier gases and the required laboratory infrastructure and consumables not used with solvents. This makes it hard to directly compare with solvent extraction long term, but would certainly make it far more expensive in the short term. It would however produce far less waste and would not require the purchasing and storage of large amounts of solvent for routine analysis. Table 3-3 shows cost comparisons between the solvents used. *t*-butyl alcohol is not currently available in the same high-grade quality as the other solvents included, but its ACS-grade equivalent still comes out as the most expensive of those tested along with HPLC-grade MTBE. Toluene and dichloromethane are the least expensive of the group, while ethyl acetate and the mixtures containing hexane fall in the middle of the price range. Disposal costs are not included, but it should be noted that,

as a chlorinated solvent, dichloromethane would likely require different disposal procedures and, therefore, may be more expensive to dispose of compared to other organic solvents. Consumable costs for TD include the use of inert sand, glass wool and the gas flows associated with N_2 sample desorption and He cold trap desorption.

Table 3-3. Costs associated with the purchase of a 4 L quantity of each respective solvent system from an identical vendor [118]. Two-solvent systems were calculated with 2 L quantities of each component solvent. Transportation, storage and disposal costs are not included.

**t*-butyl alcohol did not have HPLC-grade solvent available for purchase

	Dichloro-methane	Ethyl Acetate	Hexane: Acetone	Hexane	t-Butyl Alcohol: Acetone	Toluene	MTBE
Cost of 4 L of HPLC-grade Solvent (CAD)	245	325	280	290	480*	225	465

3.4 Conclusions

Ultimately, the most effective extraction method in all metrics aside from short-term cost was thermal desorption. Liquid extraction solvents were relatively comparable to each other analytically, with *t*-butyl alcohol/acetone showing promise as a non-targeted solvent system and likely the most effective for extractions overall. As expected, Soxhlet extraction was superior to cold shake extraction in terms of the number and intensity of compounds able to be extracted. Despite this, it is still becoming less popular as an extraction technique in industry on account of its long analysis time and large use of solvent and sample.

The recommendation would be made that for alkanes and phenyl-containing compounds the most effective solvent system for cold shake extraction is a mixture of hexane and acetone or a mixture of *t*-butyl alcohol and acetone. A higher solvent grade of *t*-butyl alcohol (if available) would likely make this a great choice of solvent system for non-targeted analysis and the extraction of hydrogen bonding compounds such as alcohols and amines. Among commonly used, high-grade solvents, ethyl acetate appears most promising for use in non-targeted analysis including alcohols, though all final solvent systems tested were relatively comparable.

Chapter 4: Ground Water Analysis of Dense Non-Aqueous-Phase Liquids by Two Streams of Extraction

4.1 Introduction

Industrial processes and extraction of natural resources can often have significant detrimental impacts on the immediate environment of a site, and in many cases this impact can be long-term and devastating [119,120]. These legacy contamination sites pose a number of challenges aside from those that could be "solved" by digging, disposing, or removing of contaminants chemically, which all pose their own issues [121-123]. These sites can also house unique opportunities to study the components of a contaminated site, allowing interesting information about the distribution, migration, depths, and even environmental weathering fates of compounds and contaminants [120,122].

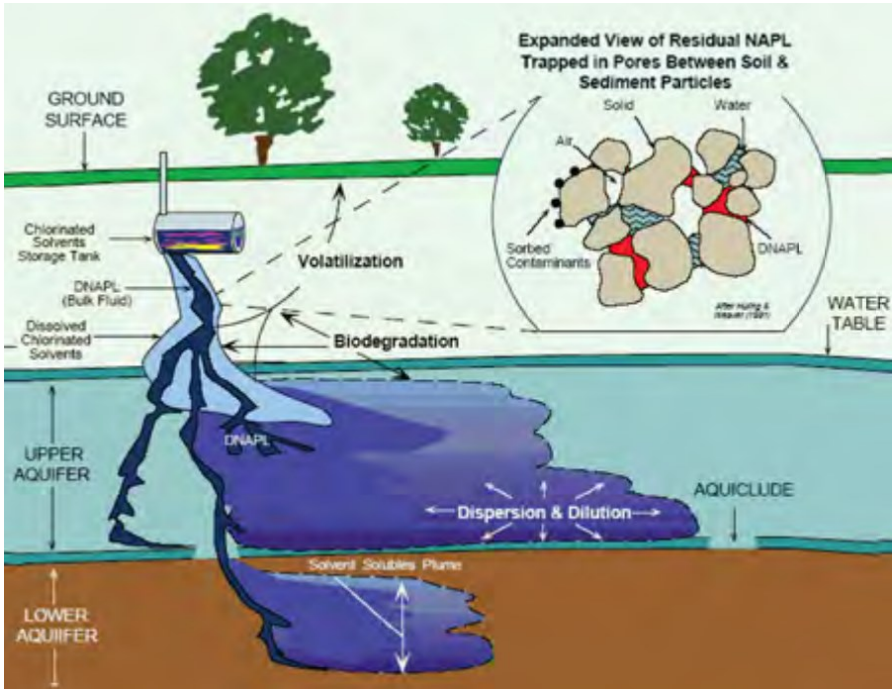


Figure 4-1. Example diagram of a contaminant source of DNAPL and how multiple levels of groundwater sources can be impacted [124].

Dense non-aqueous-phase liquids (DNAPLs) come in the form of liquid mixtures that are denser than water [119-124]. Often, there is a mixture of a number of DNAPL compounds that make up the majority of a solution, meaning that there is a potential concoction of a number of toxic chemicals that leach deep within ground water and soil sources [119-124]. Figure 4-1 shows a diagram of this from an

example contaminant source [124]. DNAPLs tend to be very difficult to remediate because of their interaction with groundwater and integration with soil and other matrices at and below the water layer [122,124,125].

Though not a comprehensive list of DNAPL compounds that can and do exist, a contaminants of concern (COC) list of toxic DNAPL compounds is included in Table 4-1. It is entirely possible, especially when considering inquiries from clients in industrial analysis, that not all compounds of interest will have standards for quantification available. To overcome this, relative quantification using calibration curves of similar response factors can be used to substitute the presence of true standards. It is important to understand and determine what factors cause response factors to change. When using an FID, the number of carbons present in a molecule should theoretically be the most important factor in determining the success of a relative quantification method [126,127].

Table 4-1. COC list that was targeted for in DNAPL analysis provided by collaborators at The University Consortium for Field-Focused Groundwater Research. "Solubility" is solubility of the listed compound in water.

Compound Class	Compound Name	MW (g/mol)	Solubility (mg/L)
Ethanes	1,1,1,2-Tetrachloroethane	168	196.8
	1,1,1-Trichloroethane	133	1290
	1,1,2,2-Tetrachloroethane	168	2830
	1,1,2-Trichloroethane	133	4590
	1,1-Dichloroethane	99	5040
	1,2-Dichloroethane	99	8600
	Chloroethane	64	6710
Ethenes	Tetrachloroethene	166	206
	Trichloroethene	131	1280
	1,1-Dichloroethene	96	2420
	cis-1,2-Dichloroethene	97	4520
	trans-1,2-Dichloroethene	97	4520
	Vinyl Chloride	62	8800
Ketones	2-Butanone	72	2.20E+05
	2-Hexanone	100	17500

	4-Methyl-2-pentanone	100	19000
	Acetone	58	1.00E+06
BTEX	Benzene	78	1790
	Ethylbenzene	106	169
	m,p-Xylenes	106	161
	o-Xylene	106	178
	Toluene	92	526
Methanes	Carbon Tetrachloride	154	793
	Chloroform	119	7950
	Methylene Chloride	85	13000
	Chloromethane	50	5320
Other	Bromodichloromethane	163	3030
	Bromoform	253	3100
	Carbon Disulfide	76	1180
	Chlorobenzene	112	498
	cis-1,3-Dichloropropene	111	2800
	Dibromochloromethane	208	2700
	Styrene	104	310
	trans-1,3-Dichloropropene	111	2800
	1,2-Dibromoethane	188	3910
	1,2-Dichloropropane	113	2800

DNAPL samples are non-aqueous, meaning that they are often not present in an aqueous sample when collected. However, the main concern of this project is with the impact of DNAPL contaminants leaching into surrounding groundwater. For this reason, samples received as DNAPL and not mixed in an aqueous solution would need to be made aqueous in a way that would resemble leaching and dissolving into water [128].

This study applies hybrid targeted/non-targeted analysis by using the separation provided by multi-dimensional chromatography to help identify their presence from a contamination source believed to be over seventy years old. The non-targeted aspect is of particular interest since DNAPL can be such a complex mixture of analytes. As only a small fraction of the overall study (with over 38 different types of analyses, ranging from microbial population estimation to dissolved gas concentration), the analysis of raw aqueous equilibrium samples of DNAPL can provide valuable insight into the highly volatile

compounds that have managed to remain at the site, and potentially be added to it over the years. Outlined in this chapter is the characterization of DNAPL and contaminated aqueous samples to better understand the fate and composition of decades old DNAPL. Relative quantification introduced in Chapter 2 is expanded upon and used to estimate the concentrations of 36 compounds often found in DNAPL.

4.2 Materials and Methods

4.2.1 Sample Reception and Preparation

Aqueous samples were received in 2 mL glass GC vials filled without headspace. Samples were left refrigerated at 7 ^{o}C until 1 hour before preparation and analysis at room temperature.

Samples that were received as non-aqueous DNAPL were equilibrated in water using a method employed by Brown et al [128]. This procedure involved the mixture of 1.0 g of DNAPL sample with 20 mL of 18.2 MΩ·cm water from a pure water dispenser (Elga LabWater via VWR International, Mississauga, ON). The mixture was made with minimal headspace in a 20 mL glass vial with a Teflon-lined screw cap (Chromatographic Specialties, Brockville, ON). Once capped, samples were wrapped in aluminum foil to keep their contents in the dark and placed on a rotary shaker for 5 d at room temperature. Once shaking was complete the samples were centrifuged for 15 min at 2350 rpm (International Equipment Company International, Needham, MA). 10 mL of the upper aqueous fraction was then transferred to a 10 mL glass vial for use in analysis.

4.2.2 Standard Preparation

A standard mix of concentrated COC compounds was made in 18.2 MΩ·cm water (Elga LabWater via VWR International, Mississauga, ON). Additions of each standard were measured to be just below each compound's level of saturation in water (Table 4-1) and added to form a solution that would be serially diluted to form a curve of each compound used in calibration. The compounds included in this standard mix were chloroform, 1,2-dichloroethane, benzene, 1,2-dichloropropane, toluene, tetrachloroethylene, ethyl benzene, m-xylene, o-xylene and 3-methyl-2-pentanone (all Millipore-Sigma, Oakville, ON).

This standard mix was used for both liquid extraction using dichloromethane and headspace extraction by SPME. None of the standards were exclusive to one of the extraction methods as none co-eluted with the dichloromethane solvent in liquid extraction.

4.2.3 Extractions Using LLE and HSPME

0.5 mL of aqueous sample was added to 9.5 mL of 18.2 MΩ·cm water (Elga LabWater via VWR International, Mississauga, ON) in a 20 mL glass centrifuge tube with a foil-lined screw cap (Thermo Fisher Scientific, Waltham, MA). 1 mL of HPLC-grade DCM was added to the 10 mL sample using a 100-1000 µL positive displacement pipette (Mandel Scientific Co. Inc., Guelph, ON). The mixture was vortexed at 100 rpm for 5 min (FroggaBio, Concord, ON) before being centrifuged at 3650 rpm for 2 min (International Equipment Company International, Needham, MA). A 1000 µL gas-tight syringe (Chromatographic Specialties, Brockville, ON) was then used to draw up 500 µL of the organic DCM layer of solution and transfer it to a 2 mL GC vial for injection on GC×GC-TOFMS/FID using splitless injection.

0.5 mL of sample was added to 9.5 mL of 18.2 MΩ·cm water (Elga LabWater via VWR International, Mississauga, ON) in a 20 mL flat-bottomed headspace vial with a PTFE-rubber crimp cap (Thermo Fisher Scientific, Waltham, MA). A 60 ^{o}C hot water bath was set up on a hot plate with a magnetic stirrer. The 20 mL vial was placed in the bath and allowed to equilibrate to temperature for 10 min. A three-phase SPME fibre (50/30 µm DVB/CAR/PDMS; divinylbenzene/carboxen on polydimethylsiloxane, Millipore Sigma, Oakville, ON) was inserted through the cap and extended into the headspace above the sample for exactly 10 minutes before retraction and transfer of the fibre to GC×GC-TOFMS.

4.2.4 Instrumentation

Analyses were performed using an Agilent 7890A chromatograph (Agilent Technologies, Santa Clara, CA, USA) fitted with an INSIGHT flow modulator operated in reverse fill-flush mode (SepSolve Analytical, Waterloo, ON). The 1D column was a 30.0 m × 0.25 mm; 0.25 µm film thickness Restek Rtx-5 (Chromatographic Specialties, Brockville, ON). The 2D was a 5.0 m × 0.25 mm; Rtx-17 phase with thicknesses of 0.25 and 0.25 µm (Chromatographic Specialties, Brockville, ON).

A 7683 series auto-injector (Agilent Technologies, Santa Clara, CA, USA) was used to inject liquid samples (1 µL) into a split-splitless injector used in splitless mode and set to 250 °C. HSPME extractions were injected manually into a SPME specific liner (Chromatographic Specialties, Brockville, ON) with the injector set to 250 °C in splitless mode.

5.0 grade He (Linde Gas & Equipment Inc., Burr Ridge, IL, USA) was used as the carrier gas at 0.73 mL/min in 1D and 15.4 mL/min in 2D. The modulation period was set to 2.3 s (flush 230 ms). A 2.8 m × 0.1 mm deactivated fused silica bleed line was used for the modulator. The oven program was 30 °C (4 min hold), 7 °C/min to 300 °C (5 min hold). After the 2D column a two-way purged splitter was used to

divide flow between an FID and mass spectrometer. The FID was operated at 300 °C with flows of 40 mL/min H_2, 250 mL/min air, and 20 mL/min makeup gas (He), respectively. The mass spectrometer was a BenchTOF-Select mass spectrometer (Markes International Ltd, Bridgend, UK), operated in tandem electron ionization mode (-12 eV and -70 eV) at 100 spectra/s in each mode. Filament voltage was set to 1.7 V, and a mass range of 40-600 m/z was selected.

Data was processed using ChromSpace® version 2.0.2 (Markes International Ltd., Llantrisant, UK). Three different processing methods were used, one for each of the two methods of detection (-70 eV MS, and FID). The -70 eV EI TOFMS processing method utilized a curve fitting algorithm with 5 pseudo-Gaussian smoothing points. A baseline offset of 0.75 s was used for the second dimension. A minimum peak area was set at a value of 5000 counts^2, with a minimum height of 2500 counts and width of 0.1 minutes. NISTMS search 2.0 and Wiley 2008 databases were used to compare with mass spectral data obtained from individual peaks. A match of 675/1000 was considered a tentative match for the identity of compounds in non-targeted/relative quantitative analysis. The FID processing method, like the TOFMS methods used a curve fitting algorithm with 5 pseudo-Gaussian smoothing points and a baseline offset of 0.75 s for the second dimension. An area of 10000 counts^2, height of 5000 counts, and width of 0.01 minutes were considered for minimum peak dimensions. Peak identities relied on mass spectral information obtained by the -70 eV (for mass spectral library identity) EI data and retention indices of target compounds.

4.3 Results and Discussion

The two extraction and injection methods complemented each other in the compounds that they were able to extract and analyze effectively. The liquid extraction and injection using DCM was most effective for compounds less volatile than DCM and provided more stable and linear quantification for these compounds (Figure 4-2). However, since DCM was an analyte of interest and was not the least volatile compound in the compounds of interest, another method was needed to analyze compounds more volatile. HSPME was able to fill this gap, accounting for the remaining 7 compounds, as indicated in Table 4-2. All samples and standards were extracted with both methods, though quantification was selected as indicated. None of the samples contained any of these seven compounds which would have all been quantified using the curve of chloroform in SPME.

Response factors of the lowest 20 µg/mL of each curve are displayed in Figure 4-2. Chlorinated alkane compounds have notably different response factors compared BTEX compounds. As would be expected

with flame ionization detection, response factor tended to depend largely on carbon number. This informed the selection of compounds for quantification in Table 4-2.

Evident from Figure 4-2 are the differences between BTEX compounds and short-chain chlorinated alkanes. The response factor is notably lower for the chlorinated compounds with three (1,2-dichloropropane), two (tetrachloroethylene and dichloroethane) and one (chloroform) carbon respectively. The curves of 1,2-dichloroethane and tetrachloroethylene are essentially superimposable in Figure 4-2. The curve of 3-methyl-2-pentanone illustrates, however, that carbon number is not the sole influence on response factor from the standards used. This compound containing six carbons was expected to have a response factor closer to that of benzene and toluene but has a lower sensitivity than 1,2-dichloropropane containing three carbons. 3-methyl-2-pentanone was therefore used only for the relative quantification of ketones with a similar carbon number.

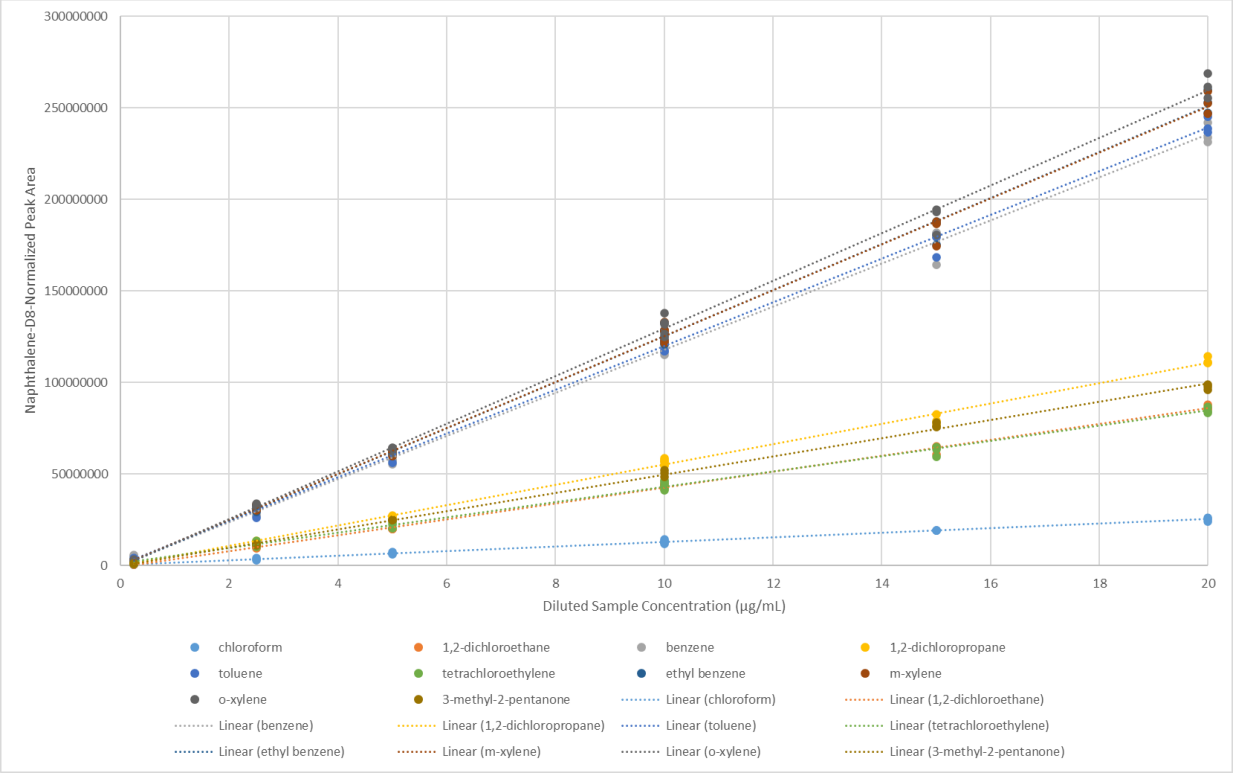


Figure 4-2. Low-intensity calibration points and curves for the standards used for quantification with FID. BTEX compounds all have higher sensitivity and very similar responses. Chlorinated compounds have response factors based on the number of carbons contained in each, as shown by the nearly identical 1,2-dichloroethane and tetrachloroethylene curves. This information was used for relative quantification of compounds not explicitly covered by standards in the calibration mix used to make this figure.

Table 4-2 indicates quantification values of each compound of interest. It should be noted that many compounds of interest were not detected in the samples and were therefore reported with a value of <LOD. Quantification values are for each compound in DNAPL samples mixed and saturated into water. Samples 003-1, 003-2, 005-1, and 005-2 were received externally and extracted directly from water samples and diluted 1/20. Samples 003-BM and 005-BM were two samples of DNAPL received previously that were mixed and prepared using the methodology of Brown et al, 2005 [128]. Results from these two samples seemed to differ to a large degree from the externally received samples prepared in the same way with samples meant to be replicates from the same sampling site.

Of particular interest of the missing compounds in Table 4-2 are the most volatile compounds (single carbon VOCs). The reasoning for this could be because they are the most volatile of the DNAPL compounds of interest. The site being measured is decades old, so it is possible that the most volatile of DNAPL components has long since seeped out. However, this theory is contradicted by the significant presence of chlorinated ethane and ethene compounds, which are also highly volatile. It is therefore likely that the contaminant source has a much higher abundance of these compounds than it does methyl compounds of a similar nature. LOD information for individual calibration curves are included in Appendix B.

Table 4-2. Quantification of compounds of interest using relative quantification methods for saturated water samples. Each compound is relatively quantified using the "curve used for quantification" in column 8 (far right). 003-1 and 005-1 were the first received of each sample. 003-2 and 005-2 were the second set received from the same two sample locations. 003-BM and 005-BM are saturated samples in water created using the long-term exposure method developed by Brown et al [128].

Compound Name	Conc 003-1 (µg/mL)	Conc 003-2 (µg/mL)	Conc 003-BM (µg/mL)	Conc 005-1 (µg/mL)	Conc 005-2 (µg/mL)	Conc 005-BM (µg/mL)	Curve used for quantification
1,1,1,2-Tetrachloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
1,1,1-Trichloroethane	1216	1155	1687	74	75	12	Tetrachloroethylene
1,1,2,2-Tetrachloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
1,1,2-Trichloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
1,1-Dichloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloroethane
1,2-Dichloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloroethane
Chloroethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloroethane
Tetrachloroethylene	149	208	630	14	13	3	Tetrachloroethylene
Trichloroethylene	175	493	865	29	31	34	Tetrachloroethylene

1,1-Dichloroethene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
cis-1,2-Dichloroethene	300	406	430	223	231	6	Tetrachloroethylene
trans-1,2-Dichloroethene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
Vinyl Chloride (chloroethene)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Tetrachloroethylene
2-Butanone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3-methyl-2-pentanone
2-Hexanone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3-methyl-2-pentanone
4-methyl-2-pentanone	62	60	25	38	37	34	3-methyl-2-pentanone
Acetone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Acetone
Benzene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Benzene
Ethylbenzene	136	187	454	54	52	24	Ethylbenzene
m,p-xylenes* (combined)	414	619	1499	139	133	61	m-xylene
o-xylene	125	155	346	60	58	30	o-xylene
Toluene	573	810	1445	228	229	34	Toluene
Carbon Tetrachloride	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Chloroform	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Methylene Chloride	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Chloromethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Bromodichloromethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Bromoform	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Carbon Disulfide	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Chlorobenzene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Benzene
cis-1,3-Dichloropropene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-dichloropropane
Dibromochloromethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	Chloroform
Styrene	7	10	8	<LOD	<LOD	<LOD	Ethylbenzene
trans-1,3-Dichloropropene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloropropane
1,2-Dibromoethane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloroethane
1,2-Dichloropropane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,2-Dichloropropane

4.4 Conclusions

Ideal quantification for environmental samples would normally be done using one extraction method, but in the case of these DNAPL samples complementary extraction methods were used to yield the best quantitative results. Liquid extraction was most effective for most compounds since it was not as limited by extraction capacity as SPME. Other compounds that eluted with or earlier than the solvent likely would have been most effectively extracted using SPME had they been present in the samples. Using these two methods complemented each other for tentative quantification of all target compounds and

provided information about untargeted compounds of potential interest. The relative quantification method proposed proved useful for quantification of compounds of interest that did not have standards. This shows the promise of this technique as a first-step screening analysis for quantification of DNAPL compounds and other VOCs.

Chapter 5: Monitoring of Microbial Degradation of Geosmin by Routine Headspace Solid-Phase Microextraction – Gas Chromatography – Triple-Quadrupole Mass Spectrometry

5.1 Introduction

A common culprit of the earthy-musty smell emitted by Streptomyces Spirulina is the compound known as geosmin. This compound, though non-toxic and an important sensory compound for humans and other organisms to locate fresh water, has been of concern for perception of quality in food, drink, and other sensory-based industries for decades [129-131]. Geosmin is commonly associated with the smell of rain and has a very low threshold of detection by the human nose at <10 ng/L [129-131]. There are few methods for the removal of geosmin from water, and the process often involves extensive filtration and pretreatment [132-134].

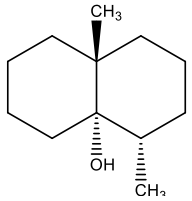


Figure 5-1. Structure of a (-)-geosmin molecule.

An alternative method of geosmin removal is biodegradation by microorganisms such as bacteria and fungi. By growing microbial communities or selected species in a culture with geosmin as a carbon source and analyzing the amount of the compounds remaining, an idea can be gained of the effectiveness and viability of biological removal methods. Geosmin (Figure 5-1) has three chiral centres, meaning that it will have 2^3 stereoisomers [135]. For early analyses in this ongoing project a pure racemic geosmin standard was spiked into samples. More recent iteration of the study looked to analyze only the naturally occurring (-)-geosmin enantiomer. This did not change how the analysis was performed since gas chromatographic separation and mass spectrometry will not be able to distinguish a resolved difference in retention time or a difference in mass spectrum between these enantiomers of geosmin. The hypothesis in this case was that the way that the geosmin was consumed, and therefore its intensity in non-control and non-t_0 samples would decrease more than it had in previous iterations of this study due to the presence of (±)-geosmin being replaced with naturally occurring (-)-geosmin. Discovering whether a microbial culture is enantioselective is novel and could even be useful outside of the scope of this study.

When developing a method of analysis for such a compound there is a fair amount of literature that focuses on the use of headspace methods, and in particular HSPME [129,130,133]. Other methods

analyzing geosmin in various matrices include enzyme-linked immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), Denaturing gradient gel electrophoresis (DGGE), and qualitatively through sensory analysis based on flavor profiling and sensory threshold [131,134]. The most common method, and the method most applicable to identification and quantification in a single analysis couples HSPME to GC-MS [129-131,134].

A fibre that is designed for targeted analysis of a compound such as geosmin would be most effective. Polydimethylsiloxane (PDMS) fibres are a logical choice due to their simplicity of structure and relative effectiveness for primarily hydrocarbon-based compounds such as geosmin [130,131].

This study is unusual for this thesis because its initial goal was to look only at geosmin as a target compound. However, the breakdown products of geosmin are of particular interest, especially if they turn out to be more toxic or have odors more problematic than geosmin itself. The characterisation of geosmin consumption breakdown products for the microbes used is also of particular interest. If the breakdown products are toxic or less desirable than geosmin itself then using the microbes for geosmin breakdown will not be viable for future commercial use. For this, GC×GC-TOFMS is used to provide a comprehensive profile of what becomes of the geosmin once it is consumed by the microbes. The goal of the project in this chapter was to design a method that could be used for the analysis of geosmin and determine three main things: whether the method would be feasible for the analysis of a variety of aqueous sample conditions; whether the stereochemistry of geosmin is important in microbial breakdown of the compound; what breakdown products resulted from the microbial breakdown of geosmin and whether or not these products are more or less harmful than geosmin itself.

5.2 Materials and Methods

5.2.1 Sample and Standard Preparation

Geosmin standards used for spiking into microbial culture samples and negative controls came in three forms. The first was a perfume grade geosmin (Perfumer Supply House LLC, Danbury, CT); the second was a racemic mixture of (±)-geosmin (Millipore-Sigma, Oakville, ON); the third was a pure (-)-geosmin standard (Millipore-Sigma, Oakville, ON). Samples were spiked with geosmin and by an external collaborator at respective start times (t_0) and left in microbial cultures for set amounts of time. Control samples were spiked with geosmin, but did not have any microbial culture added. Samples were all kept in closed glass containers in the dark at room temperature during incubation and culture growth. Sample pickup was then coordinated for a set date.

A mix of 10000 μg/mL QC standard was prepared using hexane (Millipore-Sigma, Oakville, ON), perfume-grade geosmin, benzaldehyde-d6 (Cambridge Isotope Laboratories Canada Inc., Montreal, QC), and acetophenone-d8 (Cambridge Isotope Laboratories Canada Inc., Montreal, QC) as standard compounds in ACS grade methanol (Millipore-Sigma, Oakville, ON). This solution was kept at -7 ^{o}C unless being used at room temperature to provide a small aliquot to dilute to 100 μg/mL in methanol. This second solution was kept refrigerated at 7 ^{o}C and spiked into QC standards for batch analysis.

Samples of water and microbial culture spiked with geosmin in a number of conditions were received in 10 mL screw-capped glass test tubes and left refrigerated at 7 °C until 2 hours before preparation and analysis. 2 mL aliquots of each sample were diluted with 8 mL of 18.2 MΩ·cm water from a pure water dispenser (Elga LabWater via VWR International, Mississauga, ON) in a 20 mL screw-cap glass headspace vial (Chromatographic Specialties, Brockville, ON). After aliquoting, 10 μL of 69.9 μg/mL dodecanol-d25 (Cambridge Isotope Laboratories Canada Inc., Montreal, QC) internal standard was added to each sample along with 3 g of dry NaCl salt (Millipore-Sigma, Oakville, ON).

5.2.2 Targeted Analysis Using GC-MS/MS

Water samples were received in batches and profiled using headspace solid-phase microextraction – gas chromatography – tandem mass spectrometry (HSPME-GC-MS/MS). Per batch of 10 samples, 1 quality control standard (10 μL of 100 μg/mL QC standard in 10 mL 18.2 MΩ·cm water), 1 method blank (10 μL of 69.9 μg/mL dodecanol-d25 in 10 mL 18.2 MΩ·cm water) and 2 replicates (duplicate preparations of two randomly selected samples in the batch) were prepared along with the samples.

A PAL Series I autosampler (Agilent Technologies, Mississauga, ON) was used to extract and inject samples using SPME. Headspace vials were incubated at 65 °C for 10 min, followed by extraction without agitation using a polydimethylsiloxane (PDMS) SPME fibre with a 100 μm phase thickness (Mandel Scientific Co. Inc., Guelph, ON) for 60 min. Samples were desorbed for 5 min in splitless mode into the injector of the GC at 250 °C.

Separation was performed on a Bruker 456 gas chromatograph with a triple quadrupole (QqQ) Bruker SCION mass spectrometer operated in electron impact ionization mode for detection. The GC column was a 30 m × 0.25 mm, 0.25 μm film thickness HP-5 column (Agilent Technologies, Mississauga, ON). Column flow was maintained at a constant 1.00 mL/min. The initial hold for the oven program was 40 °C for 5 min, with a ramp of 8.0 °C/min to 250 °C for a hold of 1.0 min. For analysis of geosmin the mass spectrometer was operated in MRM mode using m/z transitions of 112.0→97.1 (collision energy (CE) 12.00), 112.0→83.1 (CE 12.00), and 112.0→69.1 (CE 21.00) for geosmin. Benzaldehyde-d5 and

acetophenone-d5 compounds included in QC standard solutions was also analyzed in MRM mode, monitoring m/z transitions 111.0→82.1 (CE 6.00), 110.0→82.1 (CE 12.00), and 77.0→54 (CE 21.00). Hexane was analyzed by monitoring the ions 43 and 57.0 m/z with Q1 and Q3 run in transmission mode. Dodecanol-d25 was analyzed by monitoring 62.3, 78.3, and 94.4 m/z ions in Q1 and Q3 transmission mode (CE 7.00).

5.2.3 Non-Targeted Analysis Using GC×GC-TOFMS

Preparation for analysis on the instrument was identical to that described in 5.2.2 up to extraction of samples using a 100 µm phase thickness PDMS SPME fibre. The fibre used was instead a three-phase fibre (50/30 µm DVB/CAR/PDMS; divinylbenzene/carboxen on polydimethylsiloxane, Millipore Sigma, Oakville, ON). The autosampler used was a Gerstel Automated SPME module (Gerstel, Linthicum Heights, MD). Fibre desorption was achieved by maintaining 250 °C at the septumless head (SLH) of the Cooled Injection System-Programmable Temperature Vaporizing (CIS-PTV) inlet (Gerstel, Linthicum Heights, MD) for 300 seconds.

Separation was performed using a modified Agilent 7890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph and a Pegasus 4D TOFMS (LECO, St. Joseph, MI, USA) with a quad jet liquid nitrogen-cooled thermal modulator. The first dimension (^{1}D) column was a 5% phenyl polysilphenylene-siloxane phase (Rtx®-5MS; 60 m × 0.25 mm i.d.; 0.25 µm film thickness) connected by means of a SilTiteTM µ-Union (Trajan Scientific and Medical, Victoria, Australia) to a second dimension (^{2}D) trifluoropropylmethyl polysiloxane-type phase (Rtx-200; 1.6 m × 0.25 mm i.d.; 0.25 µm film thickness). All columns were from Restek Corporation (Restek Corp., Bellefonte, PA, USA). The 2D column was installed in a separate oven located inside the main GC oven. The carrier gas was helium at a constant flow rate of 2 mL/min. The main oven temperature program was 40 °C (5 min hold), a ramp of 8 °C/min to 250 °C (1 min hold). The secondary oven was programmed with a constant +5 °C offset relative to the primary oven. The modulation period was 2.50 s (0.40 s hot pulse and 0.85 s cold pulse time) with a +15 °C offset relative to the secondary oven. Mass spectra were acquired in the range m/z 40–800 at 200 spectra/s. The ion source temperature was set at 200 °C and the transfer line temperature was set at 240 °C. The detector voltage was run at an offset of -200 V relative to the tuning potential and the ionization electron energy (EI source) was set at 70 eV. Samples were acquired using LECO ChromaTOF® software version 4.72.0.0. NISTMS search 2.0 and Wiley 2008 databases were used to compare with mass spectral data obtained from individual peaks.

5.3 Results and Discussion

5.3.1 Concentration Change of Geosmin from Microbial Breakdown

Analyses began with exploratory studies comparing a number of different microbial spike amounts and how various mixtures of microbes were able to break down geosmin. Concentrations of 100 µg/L, 10 µg/L, and 1 µg/L were decided upon as suitable benchmarks for high, medium, and low concentrations of geosmin respectively. Additionally, these preliminary studies emphasized the importance of using pure geosmin for analyses. Figures 5-2A and 5-2B show the differences between two different spiked standards. The first in Figure 5-2A is a perfume grade geosmin standard. The second in Figure 5-2B is an analytical grade racemic mixture of (±)-geosmin. Separation and analysis techniques such as the GC-MS/MS method used, are often capable of separating diastereomers, but not enantiomers. A mirror image flowing through a narrow tube of stationary phase might as well be an identical compound. Since there are $2^3 = 8$ stereoisomers for the compound, it can be theorized that there are four diastereomers being observed, each with an enantiomer. These four subsets are therefore the four peaks observed. The purity of only one of these subsets in the (±)-geosmin explains the single peak observed in its chromatogram. This peak would theoretically be split 50/50 between the (+) and (-) in samples containing this mixture. For the (-)-geosmin standard an identical peak to that observed with the (±)-geosmin was observed, reinforcing the assertion that the separation is not enantioselective. The mass spectra for each of these isomers was identical, as shown in Figure 5-3. Intensity ratios between all four isomers were nearly identical.

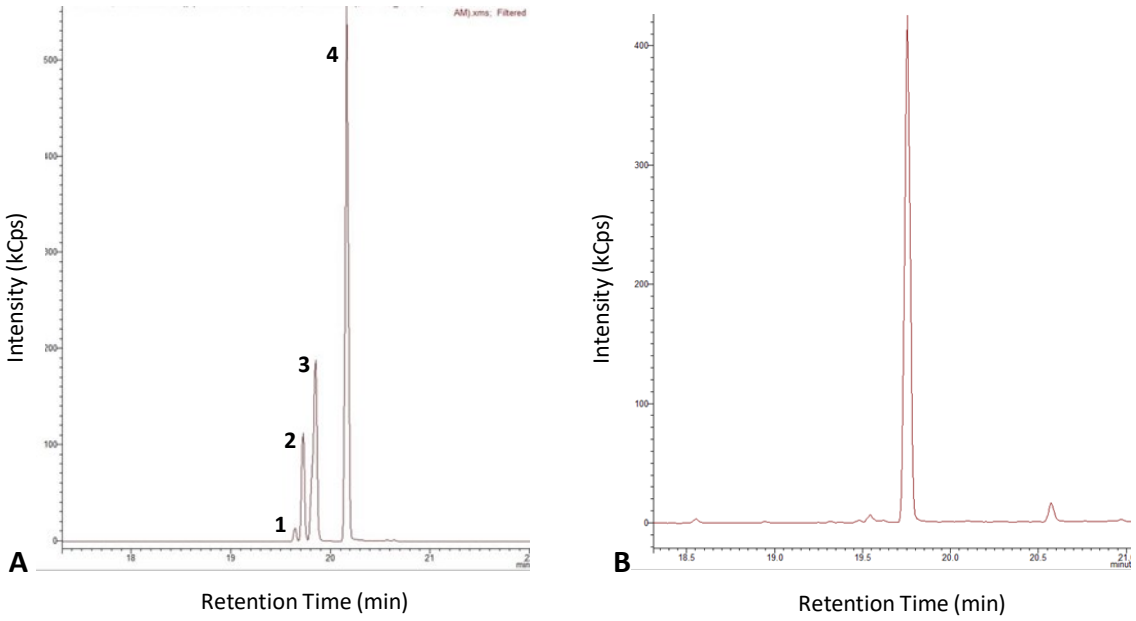
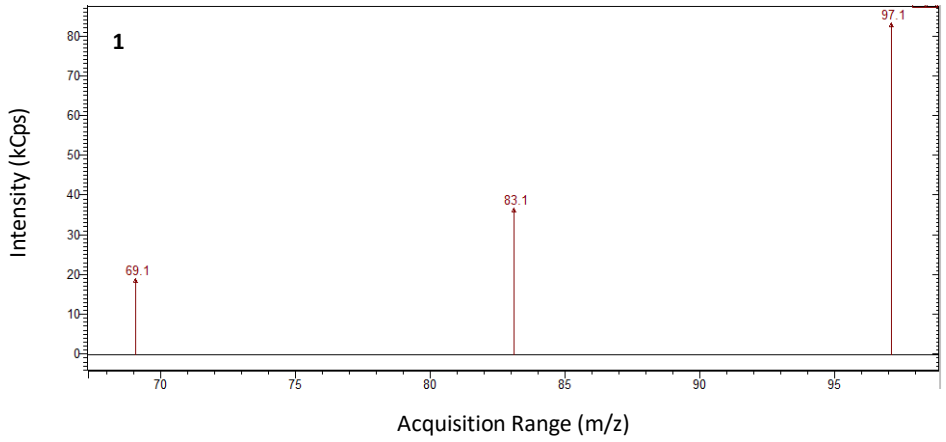


Figure 5-2. A. Four peaks present in the perfume-grade geosmin. B. Racemic (±)-geosmin with only one major peak, corresponding to the third of the four peaks observed with the perfume grade standard (A).



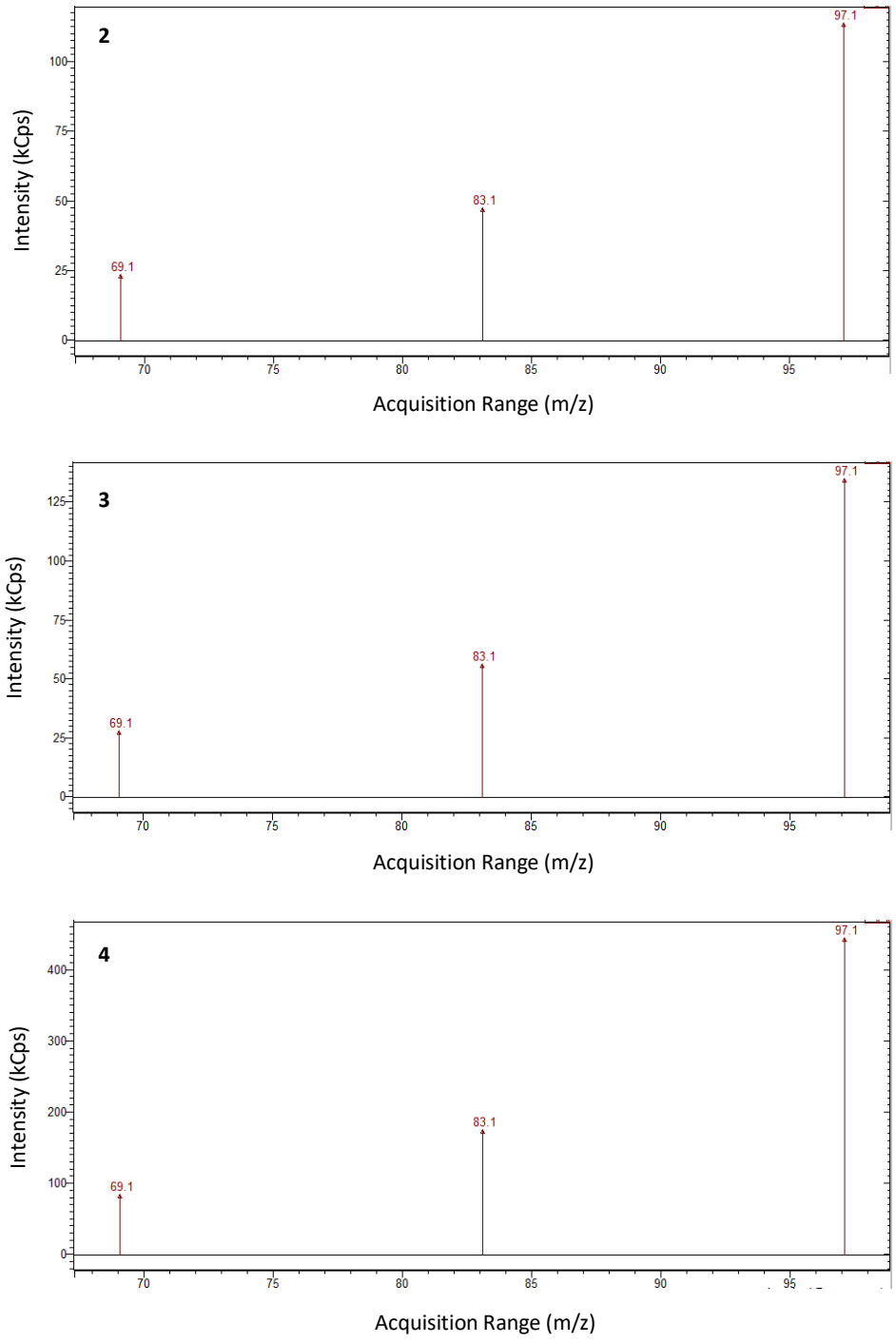


Figure 5-3. MRM spectra of each of the four diastereomers observed in Figure 5-2A. Spectra are numbered according to their labels in Figure 5-2A.

It was noticed that using the racemic mixture of (±)-geosmin, cultures were never able to completely degrade the geosmin. In fact, it appeared, as can be seen from Figure 5-4 that approximately just over half of the geosmin was being consumed by even the most effective cultures. Based on this the

hypothesis was formed that it could be just the naturally occuring (-) enantiomer of geosmin being consumed. The next step was to perform the experiment using only the naturally occurring (-)-geosmin. As can be seen from Figures 5-5, 5-6 and 5-7 the levels of (-)-geosmin remaining in samples exposed to microbial cultures are significantly lower than what was previously observed for all three concentration levels. There is almost no comparison between the negative controls and culture samples. This would lead us to believe that the microbes are very selective when it comes to the geosmin that they are consuming. They are so "picky", in fact, that they are selecting the naturally occurring enantiomer over the enantiomer that is not in a racemic mixture. Higher concentrations of geosmin (100 µg/L and 10 µg/L spikes) showed more drastic differences between negative controls and culture-containing samples compared to the low concentration 1 µg/L spike. The presumed reason for this is simply based on the concentration of the geosmin available. The more that there is for the microbial cultures to consume, they more they will consume. It is possible that the selected population of microbes are only able to consume down to approximately 75-90% less than 1 µg/L of geosmin before there becomes a scarcity of it for them to eat.

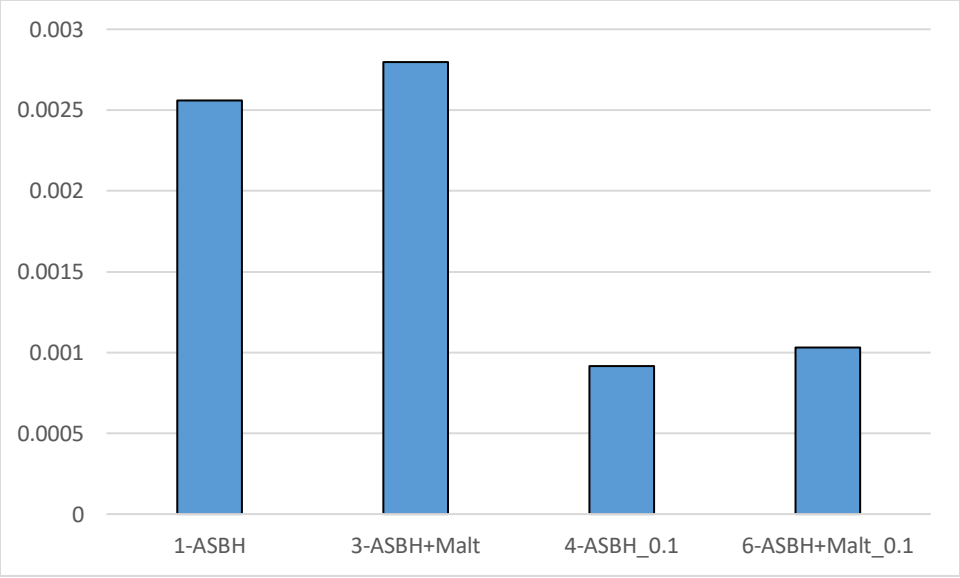


Figure 5-4. Comparison of the internal-standard-normalized intensities of samples that comprised of 10 µg/L spikes of (±)-geosmin in ASBH medium. 1-ASBH and 3-ASBH+Malt are control samples. Samples were exposed to microbial culture for 2 weeks.

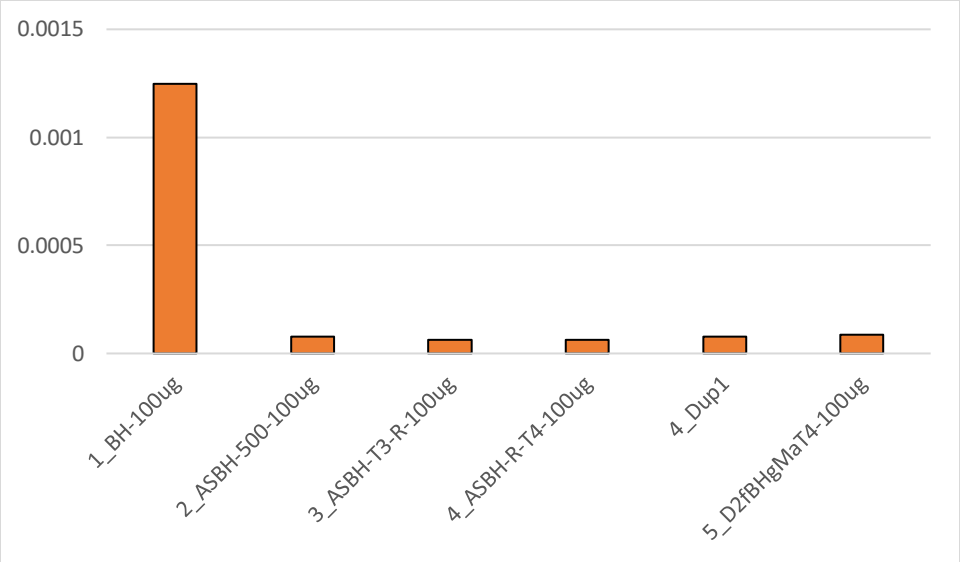


Figure 5-5. Comparison of the internal-standard-normalized intensities of samples that comprised of 100 µg/L spikes of (-)-geosmin in BH medium. 1_BH-100ug is a control sample. Samples were exposed to microbial culture for 2 weeks.

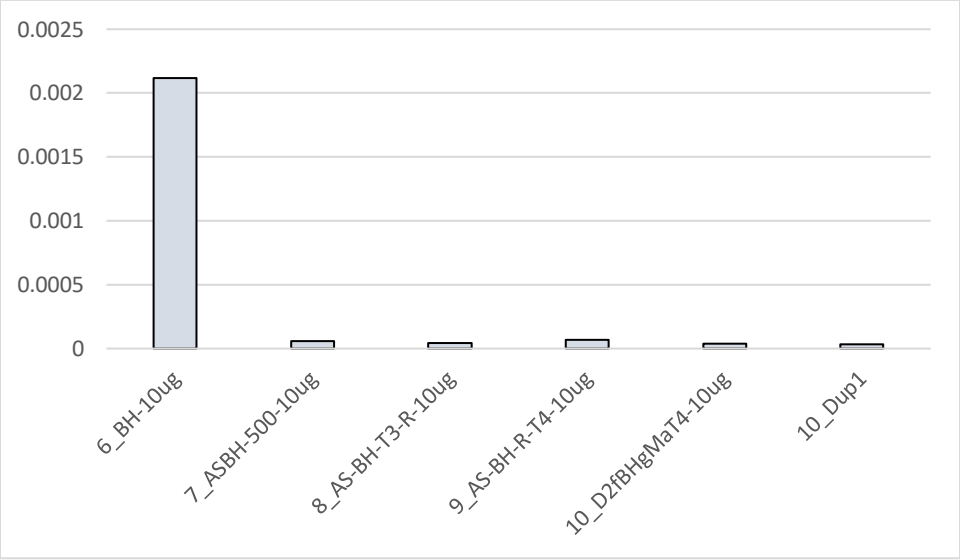


Figure 5-6. Comparison of the internal-standard-normalized intensities of Mix B samples that comprised of 10 µg/L spikes of (-)-geosmin in BH medium. 6_BH-10ug is a control sample. Samples were exposed to microbial culture for 2 weeks.

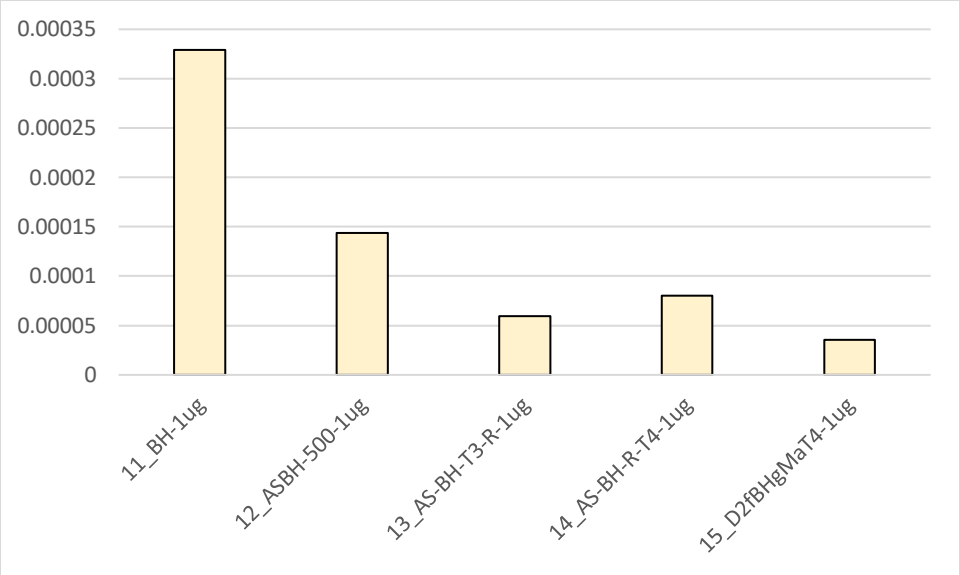


Figure 5-7. Comparison of the internal-standard-normalized intensities of Mix C samples that comprised of 1 µg/L spikes of (-)-geosmin in BH medium. 11_BH-1ug is a control sample. Samples were exposed to microbial culture for 2 weeks.

5.3.2 Microbial Breakdown Products

The fate of geosmin is after it is broken down is of great importance when considering applications for food and water cleaning. Breakdown products would need to be less toxic and non-odor producing for the breakdown process to be considered viable for use commercially. Figure 5-8 is the GC×GC-TOFMS chromatogram observed when a non-targeted analysis using three-phase SPME was analyzed. Labeled B in the figure, geosmin and its isomers are clearly shown, each peak correlating in relative intensity to those observed in Figure 5-2A. Labeled A in Figure 5-8, a number of breakdown products were also observed. These compounds were not present in a control sample that contained only geosmin in water. It is clear that mineralization of geosmin is not occurring. These compounds were tentatively identified using mass spectral library matches >700/1000. The majority of compounds identified in A were cyclic methylenes, with the most intense peak containing 1,2-dimethylenecyclohexane. Additional major peaks were identified as indene and 1-methylene-2-methylcyclohexane. Especially regarding the methylenecyclohexane compounds, they appear to be the major breakdown products of the geosmin. Mass spectral information for 1,2-dimethylenecyclohexane is included in Figure 5-9. Unfortunately, collected data for the other two main breakdown products was corrupted and unable to be accessed . Though these breakdown products do register as mild irritants and are flammable, they have fewer hazard pictograms than geosmin and are not considered toxic through ingestion or inhalation [136-138].

These compounds also tend not to have the musty odor associated with geosmin, so were a desirable result for the study.

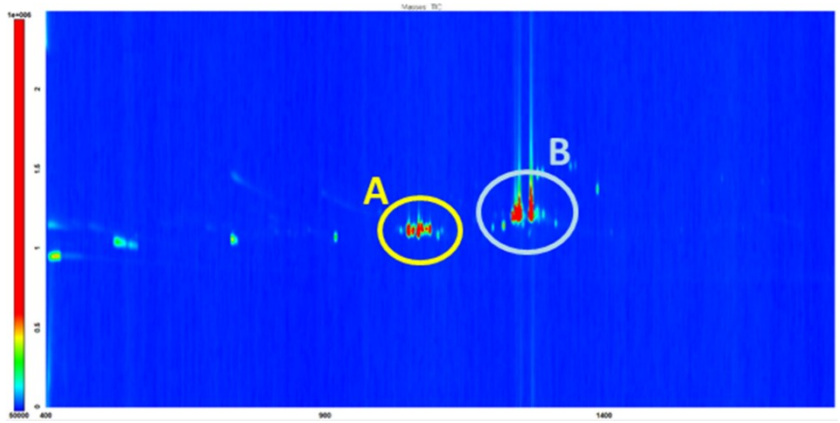


Figure 5-8. GCxGC-TOFMS TIC of a concentrated sample obtained from a bacterial culture. Geosmin and its isomers are clearly visible (B), while a number of potential breakdown products, including indenes and cyclic alkyl methylenes (A) are also visible.

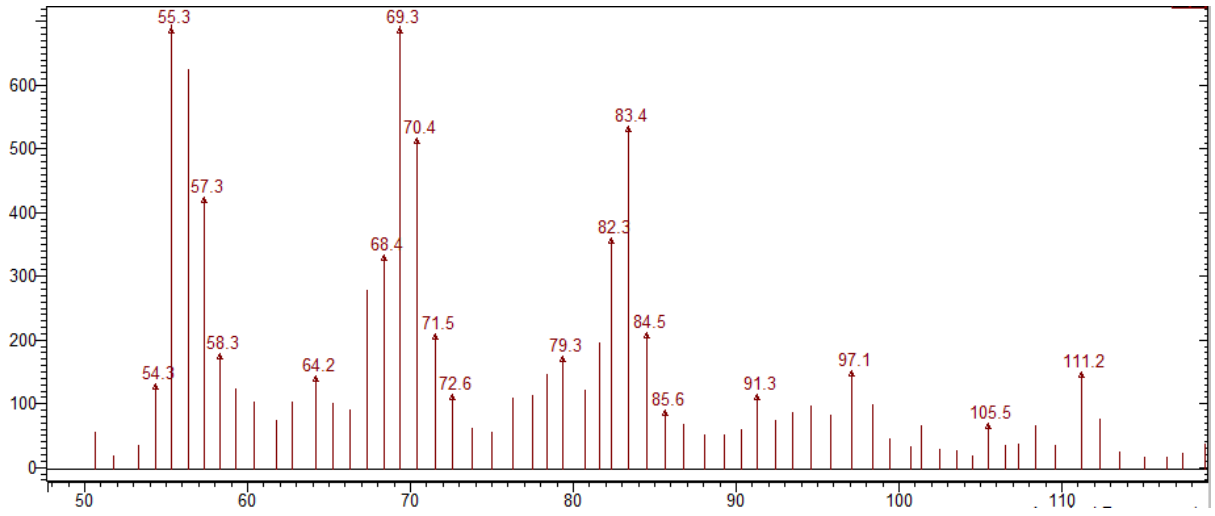


Figure 5-9. Mass spectrum for 1,2-dimethylenecyclohexane

5.4 Conclusions

Definitive results for the effectiveness of microbial degradation of geosmin were obtained. It was determined that the cultures used were very effective at breaking down specifically the naturally occurring form of (-)-geosmin. A number of relatively non-toxic breakdown products were found in a sample eaten by microbial culture. Overall, the use of microbial cultures to break down geosmin in water samples shows promise for eventual use in industry.

Next steps in this project would look at the impact on the geosmin levels if only (+)-geosmin is added to the cultures. The hypothesis here would be that the levels do not decrease compared to negative controls and t_0 samples, or only decrease very minimally.

Chapter 6: Pilot Applications for Routine Environmental Analysis

6.1 Introduction

The flashiest subjects in analytical research tend to be about creating new methods to analyze compounds. A relatively overlooked aspect of analytical research is applications. There are such a wide variety of ways to use methods that have already been developed in new and interesting ways

This chapter focuses on applications related to first response, health and agriculture. The first is a non-targeted extraction method used for the identification and quantification of degradation compounds in water when exposed long-term to water repellant fabrics. It utilizes a non-targeted liquid extraction. The second application analyzes anesthetics in theoretical wastewater from hospitals using DI-SPME. The final application was the pilot study to an ongoing long-term study analyzing volatiles in blueberries using dynamic headspace DHS.

6.2 Fabrics Aging in Water

6.2.1 Introduction

Non-targeted analyses are best utilized when there are very unclear starting points for a particular sample. This is never more true than when considering the case of a sample with unexpected need for analysis. In this study, a set of fabrics that underwent accelerated hydrothermal aging, used in a previous study by Hoque et al, 2022, were causing a colour change in the water used for aging. This prompted the question to be asked, “what is causing this?” [140]. Accelerated hydrothermal aging is normally used to help determine the deterioration of physical characteristics of fabrics; this study was done to determine potential chemical changes. Chemical changes would then be associated with the colour observed and any other changes in physical and functional characteristics of the fabrics.

Extraction solvents used for non-targeted aqueous analyses tend to have multiple characteristics that promote the extraction of compound classes [141,142]. It is impossible to have a truly universal extraction solvent, especially since the solvent needs to be immiscible with water to be separated from the sample. Non-targeted analyses of water sources using LLE have recently been demonstrated extensively with a variety of compound classes and analyses [45,143-145]. The number of distinct chemical components in a sample mixture poses a significant challenge for chemical separation [146].

An LLE involving a non-selective organic solvent lends itself well to global analysis of a wide array of compounds that would be of interest in a study such as this. A number of organic solvents could be considered to cover the array of compounds in the leached water. Ethyl acetate and dichloromethane are common for the application of non-targeted analysis in aqueous samples and other similar media [147].

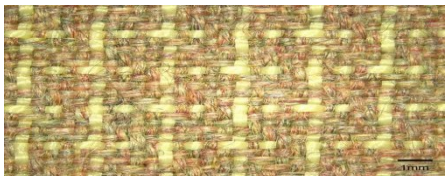
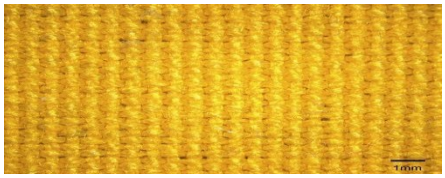
The extracts of an organic substance such as fabric would be expected to contain an array of volatile organic compounds. Due to the chemical complexity and variety expected from the degradation of fabrics and the expectation that the aqueous components of the solution would largely be semi-volatile organic compounds, GC×GC-TOFMS was deemed ideal for identification during this study. However, when it comes to quantification of organic compounds, FID remains exceptionally effective in terms of its linear range. Ultimately, a combination of two techniques was used. GC×GC-TOFMS to identify the compounds contained in a sample, and GC×GC-FID to quantify the compounds identified. The goal of this project was to design a non-targeted extraction and analysis method that could be used to characterize the chemical changes in water used to thermally age fabrics. The results could then be used by industry professionals and manufacturers to inform practices when designing fabrics.

6.2.2 Methods

6.2.2.1 Accelerated Hydrothermal Aging Process

Table 6-1. Fabric identification names, compositions and visual appearance immersed and analyzed throughout the study.

Sample Name	Fabric Characteristics	Visual Structure
MA	Composition: 64% para-aramid/36% PBI Structure: Twill weave Gram/m^2: 234 Yarns: Spun & Filaments	
MB	Composition:55% para-aramid/37% PBI/8%LCP Structure: Plain weave Gram/m^2: 246 Yarns: Spun & Filament	

MC	Composition: 65% para-aramid/35% meta-aramid Structure: Broken twill weave Gram/m^2: 222 Yarns: Spun & Filaments	
MD	Composition: 60% para-aramid/40% meta-aramid Structure: Twill weave Gram/m^2: 227 Yarns: Spun	
ME	Composition: 60% para-aramid/20% meta-aramid/20% PBO. Structure: Twill weave Gram/m^2: 226 Yarns: Spun	
MF	Composition: 93% meta-aramid/5% para-aramid/2% anti-static. Structure: Plain weave Gram/m^2: 257 Yarns: Spun	
MG	Composition: 65% para-aramid/35% PBI Structure: Twill weave Weight per surface area: 214 g/m^2 Yarns: Spun & Filaments	
MH	Composition: 65% para-aramid/35% PBI Structure: Twill weave Gram/m^2: 247 Yarns: Spun & Filaments	
SA	Composition: 60% technora/40% PBO Structure: Rip stop weave Gram/m^2: 247	

	Yarns: Spun	
SB	Composition: 65% para-aramid/35% meta-ar. Structure: Broken twill weave Gram/m^2: 244 Yarns: Spun & Filaments	
SC	Composition: 65% para-aramid/35% PBI Structure: Twill weave Gram/m^2: 219 Yarns: Spun & Filaments	

Accelerated hydrothermal aging was performed by immersing the fabric specimens detailed in Table 6-1 into reverse osmosis (RO) water for up to 50 days at 90 ^{o}C. Specimens of 10 × 20 cm were wetted with RO water in a tray by applying a 1 kg mechanical load using a stainless-steel roller. The wetted specimens were then placed in mason jars containing 500 mL 90 ^{o}C heated RO water. Separate jars were used for each fabric. All jars were then kept in an air circulating oven (Heratherm™, ThermoFisher Scientific, Ottawa, ON) for 50 days at 90 ^{o}C. One jar (Blank B) containing only RO water was placed in the oven with other fabric containing jars. Aliquots of 50 mL were collected every five days by using a volumetric pipette (Fisher Scientific, Waltham, MA) and reserved in amber bottles for further analysis. After collecting aliquots, 90 ^{o}C heated RO water was added to every jar by using a graduated cylinder to compensate for losses due to sampling and evaporation. New RO water was added every time a fabric containing jar was collected (Blank C). Thus, Blank B and Blank C aliquots were collected during the overall process to trace any potential contamination sources. Table 6-2 provides the table for potential contamination and considerations. To avoid any cross-contamination, equipment related with the aging process was washed by acetone followed by rinsing three times with RO water before using.

Table 6-2. Potential contamination and considerations accounted for by each blank for the extraction of fabric samples in water.

Contamination Source	**Accounted for by**
Jars and lids	Blank A
Oven	Blank B
Graduated cylinder	Blank B
Water source	Blank C
Amber bottles	Blank C

6.2.2.2 Liquid Extraction

Collected samples and blanks were stored in the dark at room temperature. 20 mL aliquots were taken using a 20 mL volumetric pipette (Fisher Scientific, Waltham, MA, USA) and dispensed into 45 mL glass centrifuge tubes (Fisher Scientific, Waltham, MA, USA). 0.5 mL of dichloromethane (DCM) with 4.5 µg/mL of dodecanol-d25 (both Millipore-Sigma, Oakville, ON) was injected below the sample surface using a 1 mL gas-tight syringe (Restek, Bellefonte, PA, USA). Samples were next vortexed at 1000 rpm for 5 min before being centrifuged at 3650 rpm for 2 min (International Equipment Company International, Needham, MA). 200 µL of the bottom DCM layer of the sample was transferred to GC vials with 300 µL inserts for analysis. Initial extraction trials included an identical extraction using perchloroethylene (Millipore-Sigma Co., St. Louis, MO, USA) and a modified extraction using ethyl acetate (Millipore-Sigma Co., St. Louis, MO, USA) that transferred 200 µL of the sample from a floating organic layer rather than a sinking one as was required using the other two solvents.

Dodecyl alcohol-d25 (CDN Isotopes, Pointe-Claire, QC, Canada) was used as an internal standard (IS). The IS was prepared in a solution of perchloroethylene (Millipore-Sigma Co., St. Louis, MO, USA) at a ratio of 1:2500. This perchloroethylene-IS solution was then diluted 1:100 in ACS-grade dichloromethane (Millipore-Sigma Co., St. Louis, MO, USA). This final solution in dichloromethane (with a final 1:250000 dodecyl alcohol-d25 surrogate) was used for extraction of aqueous samples.

6.2.2.3 GC×GC-FID Analysis

Samples were analyzed with a modified Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara) equipped with a four-jet dual-stage modulator (LECO, St. Joseph, MI) with a closed-loop immersion cooler and cold probe (FTS Systems, Stony Ridge, NY). Samples were injected using a 7683 autosampler (Agilent Technologies, Santa Clara, USA) into a splitless inlet set to 280 ^{o}C. Column flows were set to 2 mL/min with helium carrier gas. The first-dimension column was a 26.90 m long, 0.25 mm internal diameter, 0.25 µm film thickness Rtx-5, while the second-dimension column was a 0.830 m long, 0.25 mm internal diameter, 0.25 µm film thickness Rtx-200 (both columns from Restek, Brockville, ON, Canada). The modulation period was set to 2.50 s, with a hot pulse of 0.40 s and a 0.65 s cool time between stages. FID detector had a temperature of 315 ^{o}C with 10 mL/min helium makeup flow, 30 mL/min hydrogen flow, and 400 mL/min air flow.

The oven program for using DCM as a solvent was set to 30 ^{o}C for 4 min, then ramped 4 ^{o}C/min to 80 ^{o}C, 10 ^{o}C to 160 ^{o}C, and again 4 ^{o}C to a final temperature of 280 ^{o}C for 12 min. For initial portions of the study, ethyl acetate and perchloroethylene were also used. Ethyl acetate had a starting temperature of

50 ^{O}C with the same oven ramp rates as DCM. Perchloroethylene had a starting temperature of 90 ^{O}C, again with the same ramp rates as the other two solvents.

6.2.2.4 Mass spectral Identification

Identification using mass spectral analysis was performed using GC×GC-TOFMS. A 7683 Series autosampler (Agilent Technologies, Santa Clara, USA) was used to introduce extracted samples. The first-dimension column was an Rxi-5 phase, 30.0 m length, 0.25 mm diameter, with 0.25 µm film thickness (Restek, Brockville, ON, Canada). The second-dimension column was an Rtx-200ms phase, 5.0 m length, 0.25 mm diameter, with 1 µm film thickness (Restek, Brockville, ON, Canada). An Agilent 7890A chromatograph (Agilent Technologies, Santa Clara, CA, USA) was used and was fitted with an INSIGHT flow modulator (SepSolve Analytical, Waterloo, ON, Canada). Flows for this system were set to 2 mL/min in the first dimension and 22 mL/min in the second dimension, while the modulation period was set to 1.0 s (flush 100 ms). For both sets of analyses a 0.1 mm internal diameter bleed line for the modulator was 1 m in length. The oven program used was designed similarly to the cryogenic method in the quantitative analysis using cryogenic modulation, where the oven program was set to 30 ^{O}C for 4 min, then ramped 4 ^{O}C/min to 80 ^{O}C, 10 ^{O}C to 160 ^{O}C, and again 4 ^{O}C to a final temperature of 280 ^{O}C for 12 min. Injection volume was 1 µL, and the inlet was used in splitless mode and set to 280 ^{O}C. The chromatographic setup included an INSIGHT flow modulator and a two-way purged microfluidic splitter (both Markes International Ltd, Bridgend, UK) dividing flow to an FID and the BenchTOF. All FID analyses were performed using an Agilent 7890A FID, while mass spectrometry analyses were done in tandem (-16 eV and -70 eV) using a BenchTOF-Select mass spectrometer (Markes International Ltd, Bridgend, UK).

6.2.2.5 ChromaTOF® Processing for Quantitative Analysis of GC×GC-FID

Data was processed using ChromaTOF® version 4.71.0.0 (LECO, St. Joseph, MI, USA). First dimension peak widths were set to 15 modulations, with second dimension widths set to 0.4. The baseline offset was set to 0.8 and the expected peak widths throughout the entire chromatographic run were set to 12 s for the first dimension and 0.15 s for the second dimension. The peak-finding threshold of S/N was set to 50:1 with the minimum S/N ratio for sub-peaks to be retained set at 10. An in-house algorithm was used to identify and classify quantified peaks based on retention time. Peak tables were exported as .csv files and analyzed using in-house processing algorithms to generate data, normalize to internal standards, and produce figures.

6.2.2.6 ChromSpace® Processing for GC×GC-TOFMS

A baseline offset of 0.25 s was used for the second dimension. Version 2.0 NIST and Wiley databases were used to compare with mass spectral data obtained from individual peaks. A match of 675/1000 was considered a tentative match for the identity of compounds in non-targeted/relative quantitative analysis. A -16 eV EI TOFMS ionization channel was used to help identify a molecular ion for confirmation of library matches for 70 eV EI ionization. Peak identities relied on mass spectral information obtained simultaneously by the 70 and 12 eV EI detectors and retention indices of target compounds.

6.2.3 Results and Discussion

6.2.3.1 Solvent Selection

Figure 6-1 shows a comparison of the TICs of an identical sample extracted with three solvents. Figure 6-1A used ethyl acetate, Figure 6-1B used PCE, and Figure 6-1C used DCM. Ethyl acetate had the least comprehensive extraction of the solvents used, while DCM and PCE had notable advantages to each. Ultimately, PCE was much more effective for the extraction of alkanes, alkenes, and aromatics in the sample solution, but DCM was holistically more effective for all other compounds and was selected to better optimize non-targeted analysis.

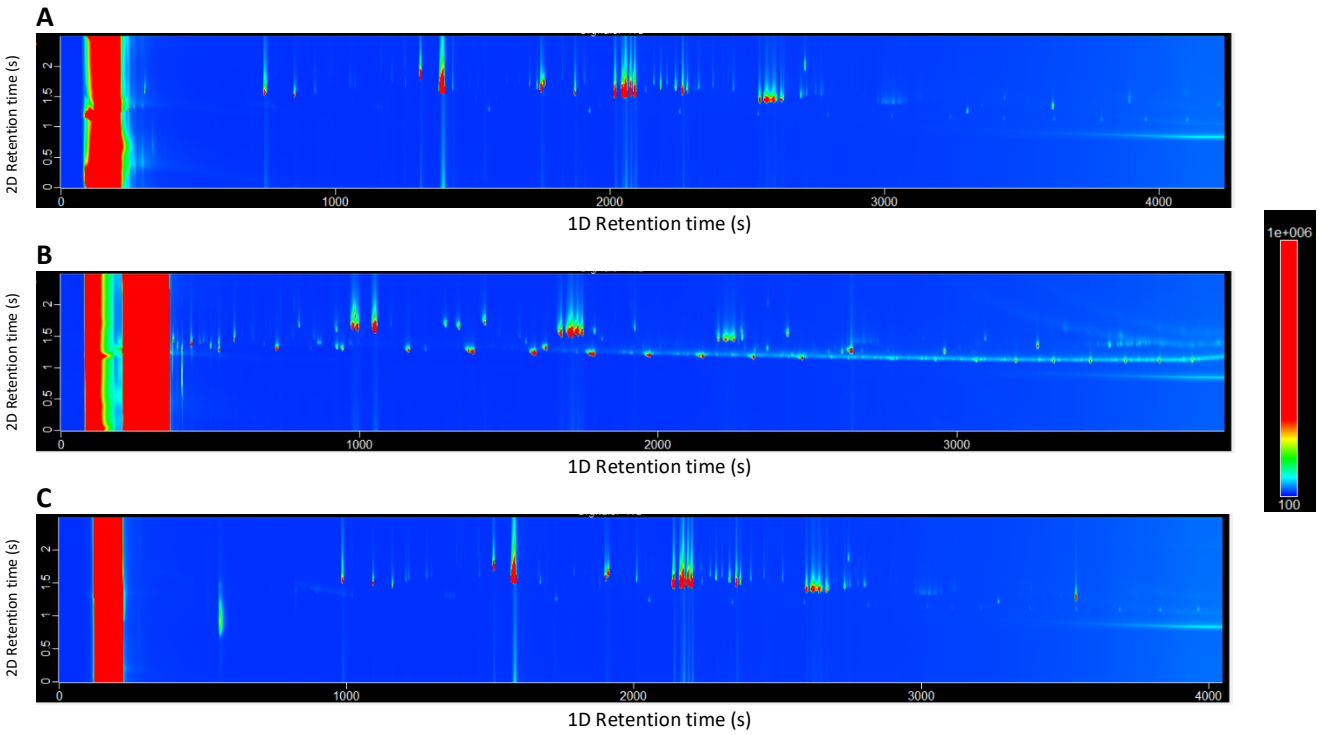


Figure 6-1. Side-by-side comparison of an identical leached fabric water sample extracted using three different solvents: ethyl acetate (A), perchloroethylene (B), dichloromethane (C). Note that the three solvents used different temperature programs, so did not have matching retention times for chromatography.

6.2.3.2 Compound Identification

Since different instruments were being used for identification and quantification, care was taken to match retention information of compounds using each instrument. GC×GC-TOFMS was used for identification with the first and last time points for each sample. GC×GC-FID was used for quantification in all 10 time points.

A large number of compounds were not tentatively identified above the set threshold of 675/1000. These compounds remained compounds of interest and were still analyzed over time for trend and intensity information. Though not in the scope of this study, the trends observed could potentially be used to help tentatively identify compounds based on similarity in trend to other compounds positively identified.

6.2.3.3 Peaks of Interest and Trends

Of interest in the thermal aging of fabric samples was what happened to particular compounds and compound classes over time. For the many compounds with higher volatility, the hypothesis was made that their concentration would be initially high and decrease over time, being lost to the equilibrium in the headspace of the open system used to heat the samples. The opposite was theorized to be true for less volatile compounds. In reality, there were too many factors in play to make any accurate predictions about how particular compounds would behave. These factors could range from those as simple as volatility of the compounds in the samples to others, such as affinity for the fabric, initial concentration in the fabric, water solubility, photosensitivity, and more. Any external factors not involving the fabrics and samples were accounted for by the blanks listed in Table 6-1.

As the study progressed it was clear, even without GC×GC-FID analysis, that a few of the samples were not equal in how they were behaving under the conditions imposed. The samples labeled MD and MF, for example produced pale brown and vibrant yellow coloration respectively in the water samples being collected. For both samples, but MF in particular, quite a few more compounds were detected and in greater abundance than other samples throughout the study. Sample SB also had a pale yellow colour to it, which was no longer visible with the naked eye after three weeks of the study. Table 6-3 compares the overall peak information for each of the samples in comparison to each other. Peaks that appeared in any of the blanks were removed from this table to ensure that only compounds from the samples were included. The number of total peaks of interest outnumbers the peaks at each (1^{st} and 10^{th}) time point because there were peaks that were detected after the first analysis and disappeared before the last analysis for each sample.

Table 6-3. Number of peaks of interest (not present in the blank) for each fabric sample.

Sample	First time point (week 1)	Last time point (week 10)	Total peaks of interest
MA	42	41	43
MB	51	49	51
MC	62	56	63
MD	98	93	100
ME	52	48	53
MF	123	115	124
MG	80	74	86
MH	69	46	71
SA	44	38	45
SB	92	83	97
SC	51	51	61

Out of 276 total compounds of interest, there was considerable overlap between each of the eleven samples. With 276 unique peaks of interest and 794 total peaks of interest combined in the samples, approximately 1/3 of peaks were only present in one sample. Figures 6-2A to 6-2C display normalized compound intensities for compounds found in multiple samples. n-butylmethacrylate is a monomer used in the production of many polymeric materials, including fabrics [148]. Octanoic acid is a naturally occurring compound often applied to fabrics to improve their appearance, feel, and performance; it is also used in the production of surfactants and detergents [149]. 1,6-diisocyanatohexane is another starting material for polymers, often used in the production of elastomers and resins in the furniture and fabric industries [150]. Each compound comparison has had its y-axis normalized to the highest intensity peak for each set. Time point 7 had an erroneously low amount of internal standard added to it during analysis accounting for the abnormally high values observed in charts. Most compounds tended to behave consistently, as seen in Figure 6-2A and 6-2B, with overall decreases or increases in intensity. Figure 6-3C shows a more interesting case where the compound of interest appears to behave differently depending on the sample being analyzed. 5 of the samples have 1,6-diisocyanatohexane decrease in concentration steadily over time, while 2 have it increasing and the other 2 are potentially staying more-or-less constant 2 more samples did not measure it at all. Referring to Table 6-1 the

increasing concentration samples have identical compositions to each other, while the two that had a constant level also had identical compositions. There were no discernable relationships between the non-present samples or the decreasing samples. Compounds that decreased over time seem to be compounds that are released by some of the fabrics and do not degrade or absorb back into the fabrics over time. These leeching compounds are of interest to manufacturers and the personnel who wear the fabrics as clothing since these are compounds that could lead to loss of functionality or exposure, particularly in the long term. Mass spectral information and library comparisons for tentative non-targeted identification of n-butylmethacrylate, octanoic acid and 1,6-diisocyanatohexane are included in Appendix B.

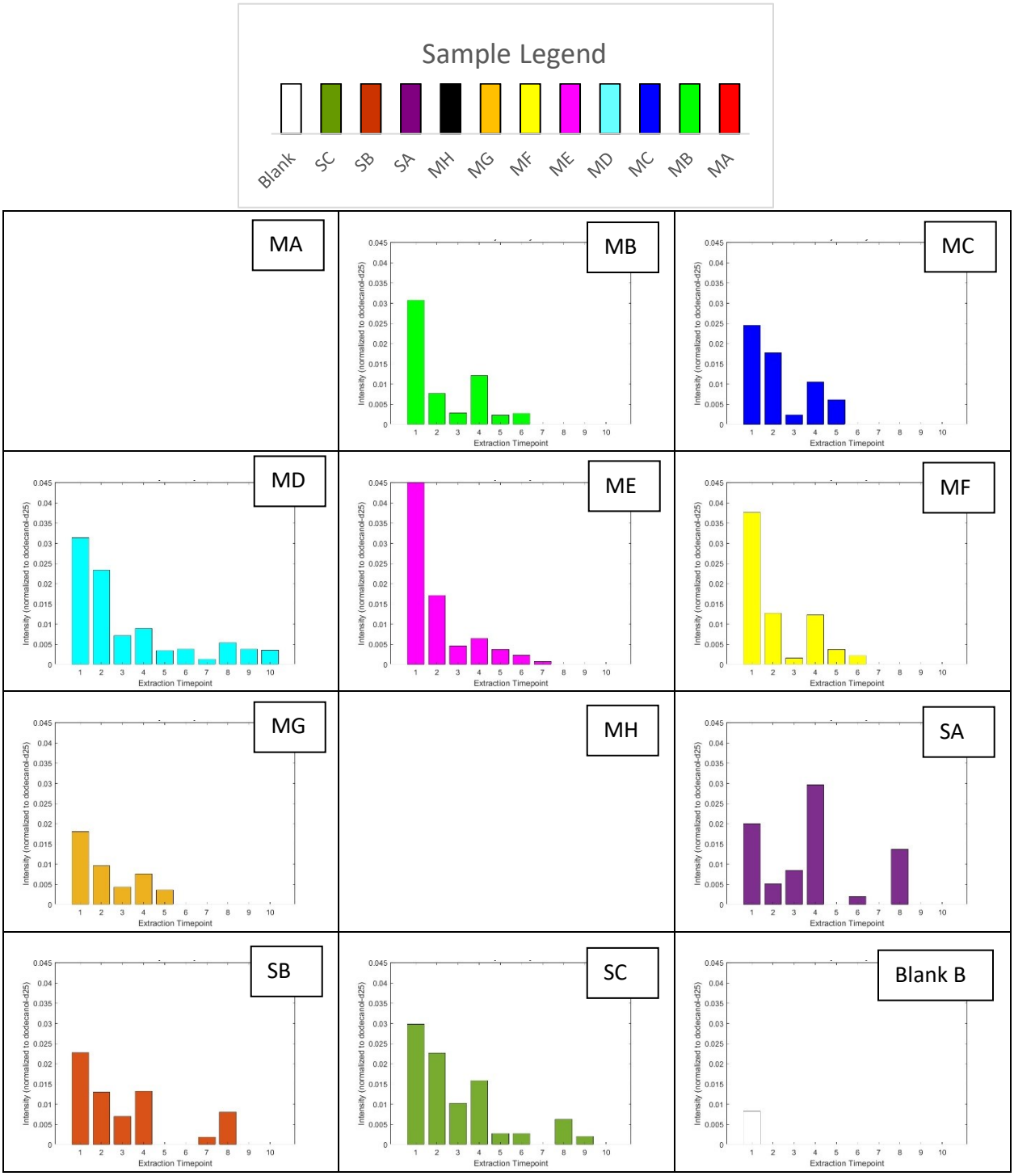


Figure 6-2A. Trends associated with n-butylmethacrylate. An example of a compound with an overall decrease in intensity in most samples, though inconsistent patterns of degradation depending on the sample being analyzed. Included in each graph is the compound's retention time and name.

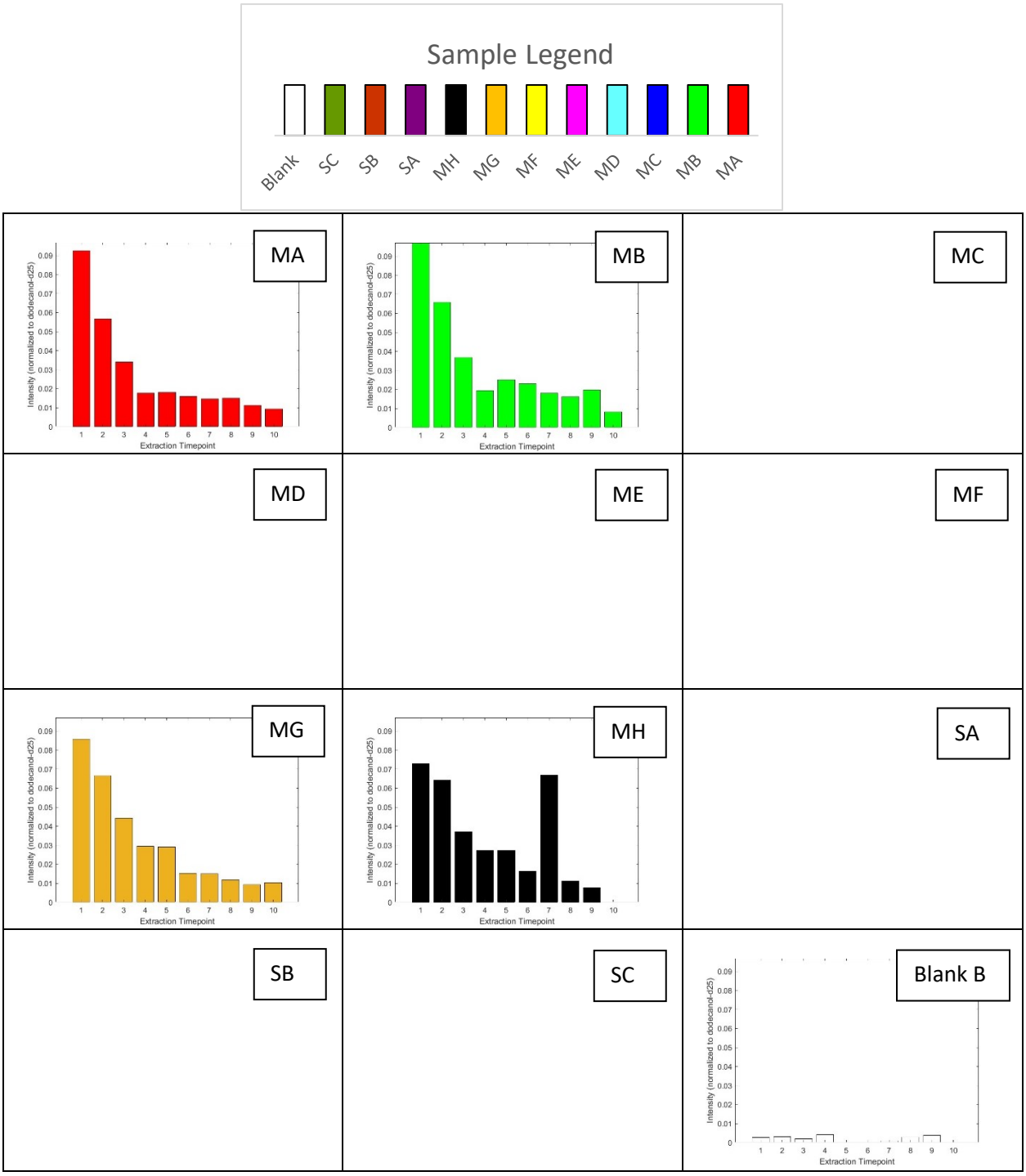


Figure 6-2B. Trends associated with octanoic acid. An example of a compound with consistent decreases in intensity over time for all samples. Time point 7 is a high outlier for all MH samples. Included in each graph is the compound's retention time and name.

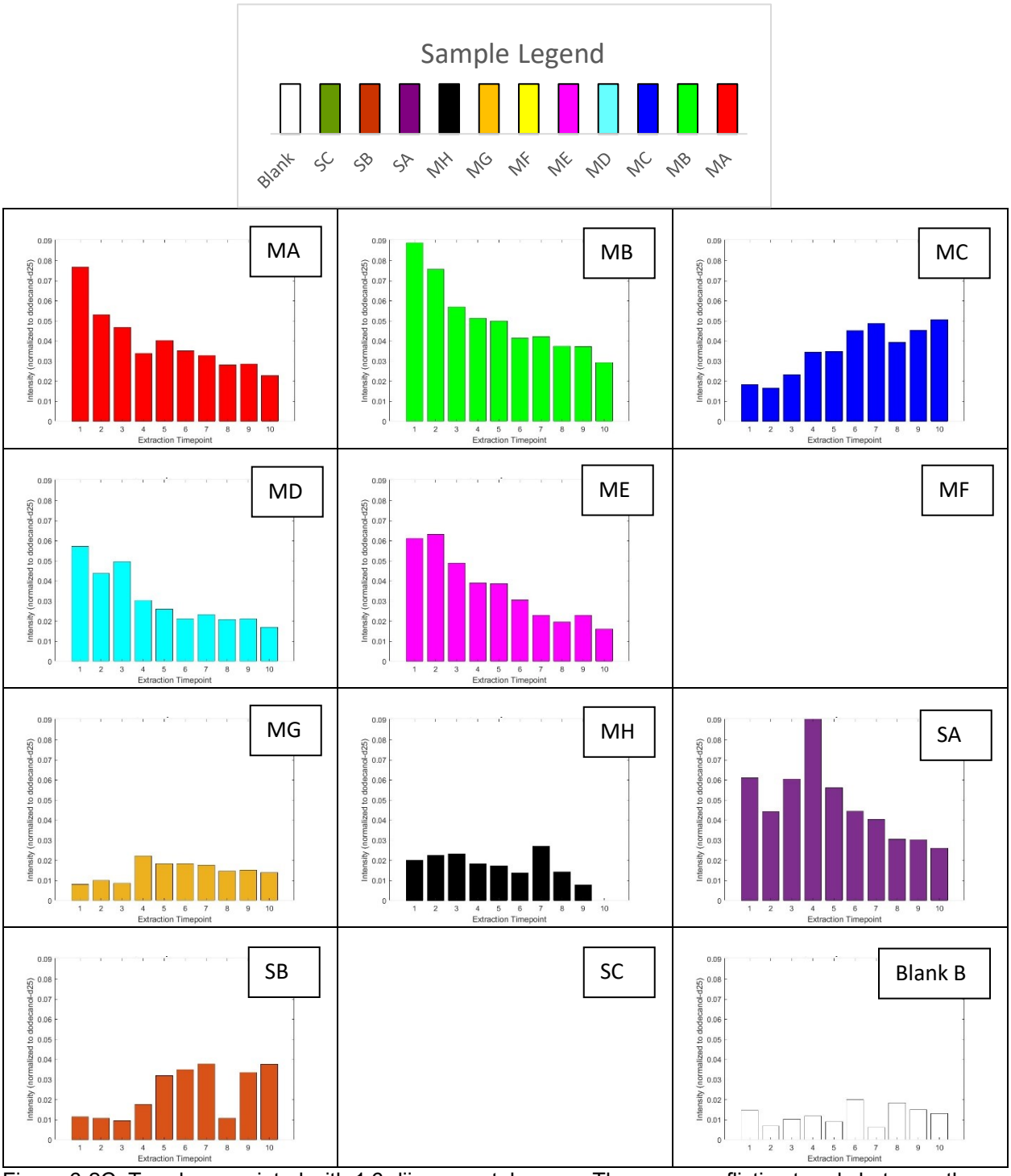


Figure 6-2C. Trends associated with 1,6-diisocyanatohexane. There are conflicting trends between the samples. MA, MB, MD, ME and SA start much higher in intensity than the other samples. MC and SB show an apparent increase in intensity, while MG and MH have decreasing trends. 1,6-diisocyanatohexane is not present in MF and SC. Included in each graph is the compound's retention time and name.

6.2.4 Conclusions

The simple 3-step benchtop extraction was very easy to apply to multiple solvent systems and samples. Samples had varying consistencies and components, but internal standard and compound intensities remained notably constant throughout the 10-week-long analysis. GC×GC-FID analysis with cryogenic modulation allowed for consistent analysis and posed no problems throughout the study.

Clear trends were observed for a large number of compounds, and the compounds identified are able to be traced to their purpose in fabric and textile production. This information gathered through non-targeted analysis can be used to inform targeted quantitative analysis of these identified compounds. It can used to inform research being done on changing physical and colour-based characteristics in the fabrics over time to shed light on what could be causing these changing characteristics. It can also be used to inform manufacturers of what compounds are being released into solution so that they can adjust formulations and production to account for their loss or to avoid their release into water systems under thorough heating and washing.

Differing trends between fabric samples, their composition and other factors show promise in determining what factors lead to different trends between samples for the same compound. This will be of interest for future work.

6.3 Aqueous Anesthetics

6.3.1 Introduction

The medical field has made incredible strides since the first use of anesthetic drugs. Having many uses from depressants to painkillers, to stimulants, the variety of drugs are undoubtedly useful and give anesthesiologists a lot to work with [151-154]. One area that the medical field seems to be given a “free pass” is in the disposal and environmental impact of the substances they use, with most research in the area revolving around illicit drugs and other drugs not used explicitly in the medical field [155-158]. Intravenous anesthetics and stimulants are among medically used compounds that have not been extensively analyzed in an environmental setting [155-158]. Given that these compounds are used daily in in medical settings to induce various reactions and anesthesia, they could conceivably induce negative impacts on organisms and systems when released to the environment, even at low concentrations [159]. It is not yet clear what kinds of effects these drugs may have on wildlife, the environment, or even the long-term health impacts on people inadvertently exposed to them through contaminated air/water [159,160].

Disposal practices in many hospitals for many of the drugs used in anesthesia is largely unregulated and is often composed of simple dumping into sinks or basins [161], even though Health Canada recommends (but has no mandates in place) to the public to not dispose of medication in this way [162]. Drainage in many Canadian cities has the added impact in some cases of direct exposure to an aquatic environment, with billions of litres of sewage entering Canadian waterways annually [163]. There is also reason to believe that metabolites of these drugs can enter the aquatic environment as components of human waste through sewage [164,165].

Microextractions are useful extraction methods when analyzing water samples while avoiding contamination of the water being sampled [166-168]. Sorbent-based microextractions are most common, with arguably the best-known being SPME [166,169]. DI-SPME and HS-SPME are both potential candidates for analysis of wastewater samples; however, the motivation behind the analysis of hospital wastewater is in search of semi-volatile and low-volatile compounds associated with anesthetic drugs used during surgeries and other medical procedures. Many of these compounds are quite large and don't lend themselves well to volatilizing into the headspace of a sample at significant concentration [170]. DI-SPME is therefore the more practical option when using GC for analysis.

SPME has been used extensively for the analysis of large and small organic molecules similar in structure to many of these intravenous drugs used in medical settings [171,172]. SPME and its multiple fibre chemistries (DVB/CAR/PDMS) are capable of extracting a wide range of different compounds depending on the chemistry/mixture of chemistries chosen. The effectiveness of a given fibre can depend on the method used. Headspace SPME (HSPME), for example, utilizes the headspace above a sample and uses concentration gradient to extract analytes. The addition of salt such as NaCl helps to induce volatilization of compounds to enter the headspace above an aqueous medium increasing the ionic strength of the solution [173]. Direct immersion SPME (DI-SPME) immerses the SPME fibre in the medium. Since the liquid medium has less separation between particles, this is often ideal to reduce equilibrium time of analytes on the SPME fibre, particularly for large molecules and drugs in complex matrices [171,174].

This chapter was conducted as a pilot study to determine how intravenous drugs can be analyzed in an aquatic environment with the use of SPME as an extraction method and gas chromatography as a separation method. A set of used anesthetics were utilized as standards for the development of an optimized method of extraction and analysis to determine potential detection limits and optimal turnaround times of analysis.

6.3.2 Materials and Methods

6.3.2.1 Standard Solution Preparation

Anesthetics and stimulants were obtained (at clinical use concentrations) from the University of Alberta Hospital. A comprehensive outline of these drugs and how they were diluted to standard mixtures can be found in Table 6-4, including the concentration received and concentration used for spiking into water samples. Water for standards and blanks was purified to 18.2 MΩ using a PURELAB flex 2 Polisher (ELGA, High Wycombe, UK). 3 g of dry NaCl was added to each standard and sample solution (Millipore-Sigma, Oakville, ON). SPME fibre assemblies and manual holders were purchased from Supelco (Millipore-Sigma, Oakville, ON). Aqueous standards were prepared by volumetric dilution of samples in 18.2 MΩ reagent water.

6.3.2.2 Extraction Process

The aqueous standard (5 mL) including an array of target analytes at 1 µg/mL of each in 18.2 MΩ water was added to a 10 mL crimp-cap vial with a PTFE-faced rubber septum. Samples were incubated in a paraffin oil bath for 5 minutes at 60 ^{o}C. Fibre housings were then punctured through the vial septum and fibres were manually extended fully into the target water medium until the top of the fibre was 1 mm below the water surface. Extraction times between 1 h and 48 h were tested. Care was taken to start and finish each extraction at exactly the allotted time (± 5 s) to minimize variability of analyte concentration.

6.3.2.3 GC-FID Analysis

Once extraction was complete, the fibre was retracted and immediately taken to the GC for analysis. The fibre was extended and desorbed by extending the fibre into an SPME liner-fitted (Restek, Bellefonte, PA, USA) inlet of an Agilent 7890 GC at 250 ^{o}C for 5 minutes. The column used was a 26.5 m, 0.25 mm, 0.25 µm Rtx-5 column (Restek, Brockville, ON, Canada). Flows were held constant at 2.0 mL/min. The temperature program of the oven was held at 40 ^{o}C for 5 min before a constant ramp of 3.5 ^{o}C/min to 300 ^{o}C and held for 5 min. Fibres were conditioned based on the manufacturer's specifications between each injection and subsequent extraction.

A standard mix of C8-C40 alkanes in hexane (Millipore-Sigma, Oakville, ON) was run in order to calculate retention indices of compounds using Kovats retention index for compound identification [175]. Additionally, samples were spiked, extracted, and run individually to aid in identification if a retention index was not conclusive for a given compound.

6.3.3 Discussion

Initial experiments indicated that HSPME took a very long time for analytes to equilibrate to the fibre. Since it still appeared that concentrations of reported analytes were increasing (i.e. not at equilibrium between the solution and headspace) HSPME was considered impractical for the application of anesthetic extraction. DI-SPME was therefore used, and optimal extraction times were tested. Sensitivity was greater, and extraction time was greatly reduced using DI-SPME. Peak areas of spiked analytes increased steadily in intensity to 24 h. 24 h and 48 h had comparable peak areas for most compounds (the exceptions being the least volatile large opioid of morphine which increased, and least volatile of phenylephrine and ephedrine which decreased), so 24 h was chosen as the best extraction time for effective analysis.

Since the drugs were obtained as used post-surgical amounts, the amount of each that could be utilized and diluted in water for analysis was sometimes low and often limited analysis. 1.0 µg/mL concentrations were used except in cases where solution volume and concentration did not allow for proper dilution. These ended up as slightly lower concentrations of 0.83 µg/mL.

Table 6-4 includes identification information of each of the compounds. Compounds that were identified with quantifiable peaks are indicated with “Yes”. Compounds identified individually, but not in the spike mix are indicated with “Yes (tentative)”. Those that were not identified at all are indicated with “No”. Remifentanil was received well past expiry, so its absence was likely due to its degradation. Hydromorphone is not very soluble in water and the least volatile of any compound included in the study and was potentially not identified based on these factors. It is unclear why glycopyrrolate was not identified.

Table 6-4. Anesthetics and stimulants received for analysis.

Name	Working concentrations received	Concentration in solution	Identified
Phenylephrine	0.1 mg/mL, 0.1 mg/mL	1.0 µg/mL	Yes
Ephedrine	50 mg/mL, 50 mg/mL	0.83 µg/mL	Yes
Neostigmine	1 mg/mL, 1 mg/mL	1.0 µg/mL	Yes
Dexamethasone	4 mg/mL	1.0 µg/mL	Yes
Propofol	10 mg/mL, 10 mg/mL	1.0 µg/mL	Yes
Rocuronium	10 mg/mL, 10 mg/mL	1.0 µg/mL	Yes
Ondansetron	2 mg/mL	1.0 µg/mL	Yes
Midazolam	5 mg/mL, 1 mg/mL, 1 mg/mL	0.83 µg/mL	Yes
Fentanyl	0.05 mg/mL	1.0 µg/mL	Yes
Morphine	5 mg/mL	1.0 µg/mL	Yes
Atropine	0.6 mg/mL, 0.6 mg/mL	1.0 µg/mL	Yes (tentative)
Sufentanil	0.005 mg/mL	1.0 µg/mL	Yes (tentative)
Remifentanil	0.05 mg/mL, 0.01 mg/mL	0.83 µg/mL	No
Glycopyrrolate	0.2 mg/mL, 0.2 mg/mL	1.0 µg/mL	No
Hydromorphone	0.2 mg/mL, 1 mg/mL	1.0 µg/mL	No

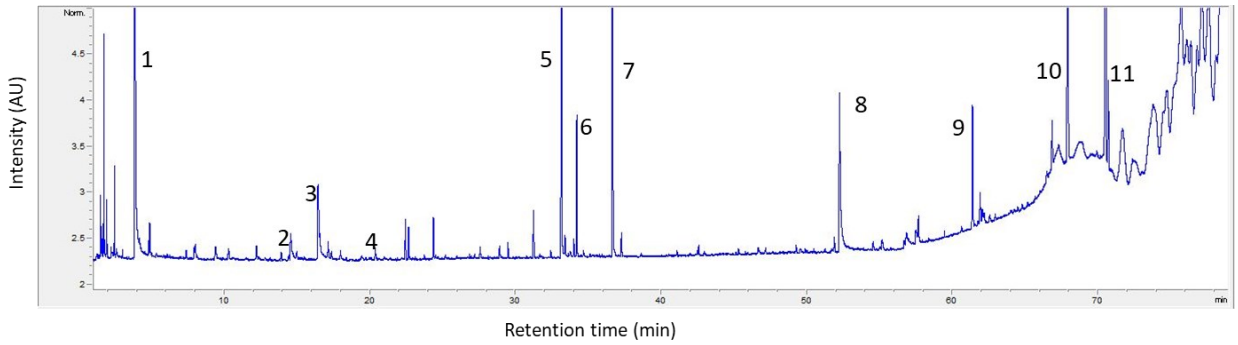


Figure 6-3. Chromatographic separation of a spiked sample of anesthetics. Compounds identities are listed: (1) phenylephrine, (2) ephedrine, (3) neostigmine, (4) dexamethasone, (5) propofol, (6) degraded rocuronium, (7) rocuronium, (8) ondansetron, (9) midazolam, (10) fentanyl, (11) morphine.

As can be seen in Figure 6-4, aside from the labeled compounds detected were a number of other peaks. These peaks were also observed in some of the individually run spikes for each of the anesthetic analytes. These are likely components of each of the clinical formulations of each of the anesthetics

since the spiked standards used were clinical samples rather than pure standards or reference materials. As a pilot study, this analysis was designed to see it the identify of compounds could be determined based on their retention information using FID. The results gained will be used to inform work done using GC-MS to provide better identification of compounds that were not identified and compounds that were not known to be present.

6.3.4 Conclusions

Though it was known what spiked standards were being analyzed this method lends itself well to application on GC-FID and GC-MS for the identification and quantification of anesthetics and other organic compounds that may be found in water systems. The 3-step extraction and analysis using 3-phase SPME fibre uses very little specialized equipment aside from the fibre itself and requires minimal manual labor from a technician or analyst. 15 out of 17 anesthetic compounds were tentatively identified well below the concentrations used in a hospital setting. Quantitative analysis could easily be done with concentrated standards of each compound, though these would likely be difficult to obtain given their highly controlled nature.

6.4 Blueberries: Fruit Volatile Analysis

6.4.1 Introduction

In the pursuit of growing and developing the highest quality product, many fruit growers have a vested interest in knowing which properties and chemical compounds contribute to the desirability of a fruit's aroma, flavour, shelf-life, and tolerance to poor growing conditions. Sophisticated chemical profiling of blueberries can, therefore, help to determine whether favourable characteristics are present at a level not perceivable by a human.

VOC profiles can be obtained easily with full automation, no added solvents, and relatively small sample sizes. HSPME has previously been applied for volatile metabolite profiling or volatilomic studies [176-185]. Although this technique has been utilized extensively, HSPME suffers from endemic drawbacks: matrix effects, selectivity due to fibre chemistry, and detection of compounds is limited to those sufficiently volatile to partition into the headspace since the method is static. The limitations of HSPME sampling may lead to analytical challenges in quantification or relative comparisons of VOC profiles, potentially causing erroneous profiles to be recovered. Instead, dynamic headspace sampling (DHS) could circumvent such issues, providing an alternative for VOC profiling [176-182].

DHS and HSPME (each of which has its own strengths for extraction and analysis) both only need a small amount of representative sample to properly profile a particular fruit. Airborne aroma profiles allow a detailed blueberry "fingerprint", which can then be used to characterize whether certain compounds are present in fruit per unit mass. Comprehensive gas chromatography coupled to time-of-flight mass spectrometry helps to maximize characterization of complex mixtures present in biological samples. The large separation capacity and high sensitivity of GC×GC and the ability of TOFMS to identify and quantify a range of library-based compounds makes them excellent for finding out what is in a sample. Here is presented a method development of automated HSPME and DHS extractions with GC×GC-TOFMS analysis of the aroma profiles of blueberries collected from local markets in Edmonton, AB.

This work was done to develop a robust protocol for blueberry aroma analysis while comparing two headspace extraction techniques and two sample preparation techniques. The proposed workflow includes sample preparation, storage, and VOC extraction. The goal was to decide which combination of sample preparation and extraction would be most suitable to a long-term study of fruit volatile components.

6.4.2 Materials and Methods

6.4.2.1 Blueberry Preparation for Headspace Extractions

Berry samples were purchased locally from the downtown Edmonton area and were stored frozen in 50 mL Nunc conical centrifuge tubes (ThermoFisher, Waltham, MA); tubes were filled (~8−12 per tube, typically). Samples were allowed to thaw at room temperature for 30 minutes prior to sample homogenization. Once thawed, whole berries were homogenized by vigorously crushing and mixing using a stainless-steel spatula to yield a homogenate of flesh, seeds, and skin. 1.2 g ± 0.2 g of homogenate was transferred to a 20 mL glass headspace vial with a screw cap and rubber Teflon-lined cap (Kimble Chase Life Sciences, Vineland, NJ) for extraction. To facilitate VOC partitioning into the headspace, 1.0 g (± 0.1 g) of salt (NaCl, Certified ACS, Fisher, CA) was added to selected vials. For the purposes of this experiment, dry salt DHS and HSPME extractions had this added. The saline solution used for the second set of DHS extractions is described: 1 mL 18.2 MΩ·cm water was measured using a 100-1000 µL positive displacement pipette (Mandel Scientific Co. Inc., Guelph, ON) and 1.0 g of salt was mixed in until saturated. Vials containing homogenate and salt/saline were vortexed for 30 s to ensure thorough mixing. Fully prepared samples were stored in the fridge at 4 °C prior to GC×GC analysis.

Quality control (QC) blueberry samples were prepared from a large batch of sun-dried blueberry raisins (Bulk Barn, Aurora, ON). QC samples were stored at 4 °C until further preparation. The QC blueberries were lyophilized using a Labconco FreeZone® Plus™ 4.5 Liter Cascade Freeze Dry System (7386021 series). Desiccated blueberry raisins were pulverized into a fine powder using a Retsch CryoMill (Retsch, Germany). Blueberry raisin powder was obtained using the following CryoMill program: pre-cooling (5 Hz, 10 minutes) followed by three milling cycles of grinding (25 Hz, 3 minutes) and intermediate cooling (5 Hz, 1 minute). QC powder was stored at -80 °C until extraction preparation. This large batch of homogeneous sample was designed to last the duration of a long-term (2+ year) study.

QC blueberry samples were allowed to thaw for 30 minutes before preparation similar to berry homogenate samples: 1.5 g ± 0.2 g of QC sample was weighed into a 20 mL vial and capped. Then, 0.6 g (± 0.1 g) of salt and 1 mL of 18.2 MΩ·cm water was added to reconstitute the desiccated powder. Vials were vortexed for 30 s to facilitate proper mixing and reconstitution. Fully prepared QC samples were stored in the fridge at 4 °C prior to GC×GC analyses.

6.4.2.2 Blueberry Water Content Determination

Samples from individual batches of blueberries (1.0 ± 0.2 g) were weighed into 28 mm tabbed aluminum dishes (VWR, Radnor, PA). Filled aluminum dishes were placed in a ventilated oven set to 105 °C for two hours to remove water without oxidation of berry solids. Dried berry solids were weighed after drying to determine the amount of water lost. Water content was calculated as a % mass for determination of dry berry mass used for extractions.

6.4.2.3 HSPME and DHS Extraction Parameters

Samples were incubated at 60 °C for 5 minutes to allow VOCs to partition into the headspace during both extraction techniques.

HSPME extraction was accomplished using a Gerstel Automated SPME module (Gerstel, Linthicum Heights, MD). A three-phase fibre (50/30 µm DVB/CAR/PDMS; divinylbenzene/carboxen on polydimethylsiloxane, Millipore Sigma, Oakville, ON) was used to extract the headspace above homogenate and QC samples. The fibre was initially conditioned according to the manufacturer's guildelines. The headspace was extracted for 20 minutes while the sample was kept at 60 °C. Fibre desorption was achieved by maintaining 250 °C at the septumless head (SLH) of the Cooled Injection System-Programmable Temperature Vaporizing (CIS-PTV) inlet (Gerstel, Linthicum Heights, MD) for 180 seconds.

DHS extraction was achieved using a Gerstel Automated Dynamic Headspace Module (Gerstel, Linthicum Heights, MD). The headspace was purged for 15 minutes (375 mL purge volume at 25 mL/min purge flow) using high-purity (4.8) nitrogen (Linde Canada Inc., Mississauga, ON). A trap temperature of 25 °C was selected to trap purged volatiles on Tenax TA adsorbent tubes (Gerstel, Linthicum Heights, MD). Adsorbent traps were further dried for 5 minutes (50 mL dry purge at 10 mL/min dry flow). Trapped analytes were desorbed by a Gerstel Thermal Desorption Unit (TDU2) and cryogenically focused by a Cooled Injection System-Programmable Temperature Vaporizing (CIS-PTV) inlet prior to GC×GC separation. Desorption of berry homogenate VOCs was accomplished in solvent vent mode with the following parameters: solvent vent time 5 min, initial temperature 50 °C (hold 5 min) ramped to 250 °C at 720 °C/min and held for 10 min with a desorption flow of 75 mL/min (helium, high-purity 5.0, Linde Canada Inc., Mississauga, ON). Cryogenic focusing of analytes was maintained in the CIS-PTV inlet, initial temperature −100 °C ramped to 250 °C at 12 °C/s (hold 3 min).

6.4.2.4 GC×GC-TOFMS Analysis

The GC×GC-TOFMS system consisted of an Agilent 7890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph and a Pegasus 4D TOFMS (LECO, St. Joseph, MI, USA) with a quad jet liquid nitrogen-cooled thermal modulator. The ^{1}D column was a 5% phenyl polysilphenylene-siloxane phase (Rtx®-5MS; 60 m × 0.25 mm i.d.; 0.25 µm film thickness) connected by means of a SilTiteTM µ-Union (Trajan Scientific and Medical, Victoria, Australia) to a ^{2}D Rtx-200; 1.6 m × 0.25 mm i.d.; 0.25 µm film thickness. All columns were from Restek Corporation (Restek Corp., Bellefonte, PA, USA). The 2D column was installed in a separate oven located inside the main GC oven. The carrier gas was helium at a corrected constant flow rate of 2 mL/min and the injector operated in solvent vent mode. The main oven temperature program was 40 °C (3 min hold), a ramp of 3.5 °C·min-1 to 190 °C (no hold), and a final ramp of 15 °C·min-1 to 290 °C (12 min hold). The secondary oven was programmed with a constant +5 °C offset relative to the primary oven. The modulation period was 2.50 s (0.40 s hot pulse and 0.85 s cold pulse time) with a +15 °C offset relative to the secondary oven. Mass spectra were acquired in the range m/z 40–800 at 200 spectra·s-1. The ion source temperature was set at 200 °C and the transfer line temperature was set at 240 °C. The detector voltage was run at an offset of -200 V relative to the tuning potential and the ionization electron energy (EI source) was set at 70 eV. Samples were acquired using LECO ChromaTOF® software version 4.72.0.0.

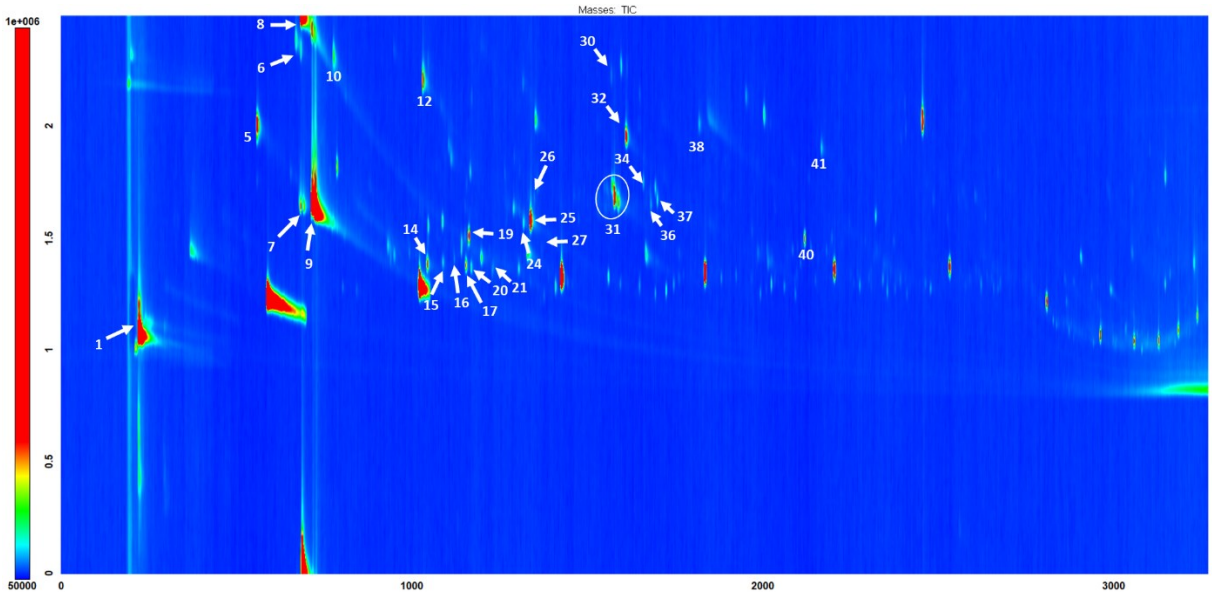
6.4.3 Results and Discussion

Comparisons were made between the number of total peaks observed using the three extraction types. Since the DHS and SPME methods were optimized to maximize the number and intensity of peaks any differences in the results would come from the extraction types themselves. Table 6-5 indicates a comparison between the extraction setup tested and the number of total peaks and peaks of interest observed for each. Peaks of interest include terpenes, ketones and aldehydes that contribute to the aroma profile of a blueberry sample. Overall profiles between DHS and SPME samples extracted with dry salt added are included in Figures 6-5A and 6-5B. The compounds of interest that were tentatively identified are listed in Figure 6-5C.

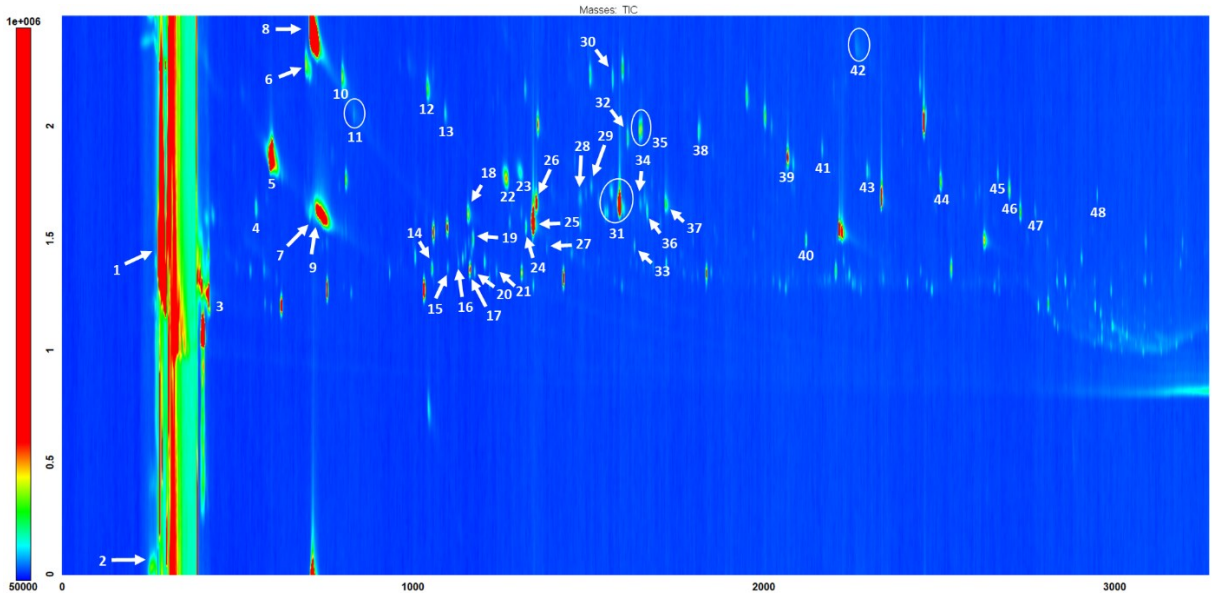
Table 6-5. Extraction performance in terms of total peaks and peaks of interest for the three extraction setups used for determination of volatiles in locally obtained blueberry samples.

Extraction Setup	Total Peaks	Peaks of Interest
SPME (+ dry salt)	1064	33
DHS + saline	860	42
DHS + dry salt	1255	48

A



B



C

1. Acetone
2. Acetaldehyde
3. Acetic acid
4. Methyl isovalerate
5. Hexanal
6. (Z)-2-Hexenal
7. (Z)-3-Hexen-1-ol
8. (E)-2-Hexenal
9. (E)-3-Hexen-1-ol
10. 2-Heptanone
11. Heptanal
12. 6-Methyl-5-hepten-2-one
13. Octanal
14. β-Myrcene
15. α-Terpinene
16. α-Terpinolene
17. Limonene
18. 2-Ethyl-1-hexanol
19. 1,8-Cineole (Eucalyptol)
20. (Z)-β-Ocimene
21. (E)-β-Ocimene
22. Linalool oxide A
23. Linalool oxide B
24. p-Cymene
25. Linalool
26. Hotrienol
27. 1,3,8-p-Menthatriene
28. (Z)-Ocimenol
29. (E)-Ocimenol
30. Limonene oxide
31. α-Terpineol Isomers
32. Decanal
33. α-Ionene
34. cis-Carveol
35. p-Menthenal Isomers
36. Santolina triene
37. Geraniol
38. Undecanal
39. Dodecanal
40. β-Caryophyllene
41. Geranyl Acetone
42. Levoglucosan
43. Tridecanal
44. Tetradecanal
45. 2-Pentdecanone
46. Pentadecanal
47. Diisobutyl phthalate
48. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione

Figure 6-4. Labeled chromatograms of a blueberry sample. A. Extraction using SPME and salt. B. Extraction using DHS and salt. Peaks of interest are labeled according to the numbers provided in C.

The overall profiles differed between SPME and DHS, particularly for the most and least concentrated compounds in the sample. As can be seen with samples 8 and 9 in Figure 6-5, (E)-2-hexenal and (E)-3-hexen-1-ol were in high abundance using both methods. This is exaggerated in the SPME extraction. The limited sorbent capacity of the SPME fibre and competition between compounds would favor those in highest abundance, leading to their dominance in the chromatogram. This is in contrast to the lower abundance peaks and less volatile compounds, such as those numbered 42-48. These compounds were far lower in abundance using SPME and are not even detected in the sample shown. Comparisons between the saline solution and dry salt addition were similar in overall profile. The only difference being the higher intensity and higher number of visible peaks with the dry salt.

6.4.4 Conclusions

The extraction setup that yielded the largest number of compounds, but more importantly the largest number of compounds of interest, was the DHS extraction with dry salt added and mixed in. This makes it ideal for use in a long-term analysis of the fruit being analyzed. This protocol, as designed here, is still being used in an ongoing blueberry analysis project as of Jan 2023. More details of this long-term study and finalization of the method can be found in the recent publication by Dias et. al. 2022 [186].

Chapter 7: Conclusions and Future Work

7.1 Conclusions

As discussed throughout this thesis, the opportunity remains to update many routine analysis methods used in industrial environmental analysis. Discussed throughout were examples of ways to take advantage of this opportunity, both by updating/providing alternatives to existing methods and by creating entirely new applications for existing methods of extraction and analysis.

Acceptable levels of TEH and TPH in water and soil range from 0.04 μg/L to 0.5 μg/mL depending on the analyte in question [187]. The majority of methods measuring TEH and TPH in routine analysis tend to group the two classes together and report them with a collective detection limit, often close to the concentration of those compounds on the high end of the spectrum (0.5 μg/mL) [5]. As a result, the current methods being used in routine do not come close to meeting the needs of the industry or society as a whole.

The application of a benchtop DLLME method to supplement the use of a common LLE method in **CHAPTER 2** shows how one particular new method could be used in a way that would require little infrastructural change. Having been conceived in 2006, this method did not exist when many routine TEH and TPH extraction methods were developed in the late 1990s. The results showed benefits not only in targeted analysis (of immediate interest to the current workflows), but also showed the potential for non-targeted analysis. **CHAPTER 2** was a comprehensive look at what it would take to consider all aspects of a method to be adapted for use in a contract laboratory. It was very effective in providing insight to why method development a contract laboratory can be difficult to accomplish. Quite a number of parameters needed to be tested and compared; changing these parameters in a "time is money" type of lab takes up that precious time and money. If the methods being used are "good enough", it is financially viable to continue to use them and forego innovation. This, of course, causes the industry as a whole to stagnate. This is why it is so important for methods being developed to have the industry using them in mind.

The hybrid targeted-non-targeted analysis introduced in **CHAPTER 2** in part inspired a more in-depth use of relative quantification in **CHAPTER 4.** Here, tentative quantification was possible for 36 compounds when only 10 standards were available. Whether this quantification is valid and what will be done to validate it is discussed in **Section 7.2.3.**

CHAPTER 3 was designed (aside from the application of thermal desorption) to be run in tandem with routine analyses already being run, cutting costs and instrument downtime while performing method development. The testing of new solvents used nearly identical methods, meaning that changes between samples would not need to be made and statistical analysis could be the main driver in determining the success of an extraction system. Results showed that there is potential for lesser used extraction solvents, such as *t*-butyl alcohol act as valuable alternatives to the solvents currently being used in routine analysis and showed the viability of a range of different solvents currently used in the industry.

One takeaway from **CHAPTER 3** was the overwhelming performance from thermal desorption, not only analytically, but environmentally. Even though the sample sizes and extraction parameters could not be directly contrasted, it showed that it would be the clear choice for any lab wanting to do volatile and semi-volatile analysis in soil and shale long-term. One aspect that was not tested was whether there were issues for the analysis of heavy hydrocarbons and similar analytes using thermal desorption. It is anticipated that there could be issues desorbing from the cold trap and other sorbents for these large molecules with slow kinetics. As a result, particular samples could cause issues that would not be as detrimental using liquid extractions.

CHAPTER 5 gave an example of a study from start to finish that effectively utilized a routine analysis method that without modification was able to adjust to new sample types, concentrations and yield exciting results. The method was developed and tested over a period of two weeks, then was not changed for a period of two years while a regularly received list of samples utilized it. The results obtained have been able to inform actionable items (**Section 7.2.4**) that will be very interesting to see the impact of long-term.

Further to **CHAPTER 5** was the work done in **CHAPTER 6.** In particular was the work with the leaching of fabrics info water over time in **Section 6.1** which has also been able to inform action items on the part of a collaborator (**Section 7.2.5**). As in **CHAPTER 5** the method was designed and completed with the sample in mind, and as with **CHAPTER 3** a solvent was selected to best perform the non-targeted analysis desired. **CHAPTER 6** also included the development of a fruit analysis method (**Section 6.3**) which has already had further application that will be discussed in **Section 7.2.5**.

7.2 Future Work

7.2.1 DLLME in Water

The analysis of water itself is a far-reaching topic, and the natural progression of this analytical method would be to explore applications. The first of which would be to analyze melted snow for the presence of environmental contaminants. As the sampling regions in Figure 2.1 show, the extraction method could be used on water samples taken from almost anywhere.

Further modifications for the method would probably involve user-friendly modifications, such as the use of centrifuge tubes with a narrower tip to make the collection of extractant easier after centrifugation, as well as the use of standardized volumetric pumps to administer solvent, which would streamline the method even further in the extraction stages. These modifications could be tested for repeatability in relation to the method developed in **CHAPTER 2**.

7.2.2 Database Creation for Non-Targeted Extraction of Soil and Shale

One piece of information that was missing to a degree was holistic non-targeted analysis information. The use of scripts to identify alcohols is a great start, but developing them to the point where they can confidently identify all hydrocarbon derivatives and functional groups would be instrumental in providing fast identification information to labs that do analysis using GC-MS and GC×GC-MS.

Additionally, creating a database of standardized statistical comparisons of different solvent systems and extraction types would be a great way for routine analytical labs to choose next steps for a given analyte and extraction method template. In this way they could alter their catalogue so that a client could ask for a particular analyte of interest, class of interest, or non-targeted analysis instead of the lab telling a client what they can measure for them.

7.2.3 DNAPL Extraction and Analysis

The results shown in **CHAPTER 4** are part of a large-scale study on the DNAPL contamination of a 60+ year old groundwater site in south-central Ontario. Other aspects of the study include remediation efforts, microbiological studies and more.

Validation of the work done to relatively quantify the compounds of interest would be another next step that could be done with a single study. As shown in **CHAPTER 4**, it is possible to use the method of relative quantification proposed to make a reasonable approximation of the concentration of compounds in solution. The proposed study would require the acquisition of a standard mix containing

all 36 of the compounds of interest in Table 4-1 and the development of spiked "unknowns" with known amounts of each of the compounds in the mixture. Calibration curves would be made, as done for Figure 4-2, which could have their response factors compared in the same way done in **Section 4.3.** A table could then be made to replicate Table 4-2, with the addition of a column quantifying each compound using individual calibration curves for each. The hypothesis of this project would be that relative quantification functions to gauge the real concentration of a component analyte in a mixture within the standard deviation of the real value. If true, this could open the door for quantification like that done in **CHAPTER 4** to be done in the future with far more confidence.

7.2.4 Geosmin Analysis by HSPME

This project reached a completion point in November of 2022 with the confirmation of the hypothesis that (-)-geosmin is particularly desirable for consumption by microbes in culture. Breakdown of geosmin in water samples has been concluded to be effective. Next steps involve the removal of geosmin from water samples entirely (or almost entirely).

Fortunately for the side of analytical method development, the geosmin analysis method is complete. Tests are already underway to determine whether small, organic microbeads will accelerate the breakdown of geosmin by giving the microbes a more definite foundation to grow. A similar hypothesis is being tested with the addition of vegetable oils.

7.2.5 Completed Pilot Studies

With the results obtained from the work done in **Section 6.1**, work in the field of human ecology is already being done to report deliverables to the manufacturers of the eleven different fabric types used. Their expertise in the field and knowledge of the fabric components (which is usually kept proprietary) along with the information included in the upcoming publication on the work will lead to the development of more leach-resistant materials. Additionally, it provides information about what compounds are taken up by the fabrics over time, which can provide important toxicological information. This is especially important for clothing that is worn long-term, which can be a passive source of chemical exposure if certain types of fabric are more prone to chemical absorption than others. Focused testing of which type of fabric is most effective at absorbing toxic aqueous components would help to address the concerns raised in the previous paragraph. This could be organized by performing a similar additional test to that done in **Section 6.1**, whereby a solution with spiked compounds of concern could be made in water and each of the fabric samples could be exposed to

them. They would then be extracted using solvent or thermal desorption and analyzed by GC×GC-TOFMS for the presence and quantity of each of the spiked compounds.

The project involving anesthetics in wastewater unfortunately lost contact with its main collaborators at the University of Alberta Hospital during the start of the COVID-19 pandemic. Were this project to continue it would look to acquire real samples from the sinks and wastewater of the hospital. This would require custodial help and likely its own ethics approval, but the determination of aqueous anesthetics that make their way to the hospital's (and the city's) wastewater would give valuable insight to the toxicological impact that the common practice of disposing of anesthetics into wastewater has on the waterways of a city like Edmonton.

Last to mention is the work done with blueberries. As mentioned in **Section 6.3.4**, this "future work" is actually current work. This routine analysis by DHS has been a long-term, large-scale project for over two years at this point, with hundreds of samples being successfully run. Modifications for the method for the analysis of blueberries are unlikely. However, use of the method or a slightly modified one for other agricultural fruits is highly likely and the protocol proposed would certainly be viable.

References

1. Muscalu, A.M.; Górecki, T. *Trends in Anal Chem*. 2018, 106, 225-245.
2. Nizio, K.D.; McGinitie, M.M.; Harynuk, J.J. *J of Chromatogr A*. 2012, 14, 12-23.
3. Picó, Y.; Campo, J. *Mass Spec Food Env Chem.* 2022, 17, 1-23.
4. Singh, S.; Ashesh, A.; Devi, N.L.; Yadav, I.C. *Microchem J*. 2022, 183, 108005.
5. Canadian Association of Laboratory Accreditation, CALA Directory of Laboratories https://directory.cala.ca/directory-search, 2022 (accessed 30 July 2022)
6. Kann, M.E. Environmental Democracy, in: Environmental Democracy, 1986.
7. Braverman, H. The Modern Corporation, in: Labor and Monopoly Capital: The Degradation of Work in the Twentieth Century, 1998.
8. Online Etymology Dictionary, Chromatography. https://www.etymonline.com/word/chromatography Edited Feb. 15, 2019. Accessed Dec. 27, 2022.
9. Nelson, D.L.; Cox, M.M. Lipids, in: Principles of Biochemistry. 6th Edition (2013).
10. Harris, D.C.; Lucy, C.A. Gas Chromatography, in: Quantitative Analysis, 10th Edition (2020).
11. Snow, N.H.; Slack, G.C. *TrAC Trends in Anal Chem.* 2002, 21 (9-10), 608-617.
12. Sugita, K.; Sato, H. *Anal Sci*. 2021, 37 (1), 159-165.
13. Paiva, A.C.; Crucello, J.; de Aguiar Porto, N.; Hantao, L.W. *TrAC Trends in Anal Chem.* 2021, 139, 116252.
14. Grob, R.L.; Barry, E.F. Theory and Basics, in: Modern Practice of Gas Chromatography. 4th Edition (2004).
15. Purnell, H. Solution, Adsorption and Partition, in: Gas Chromatography. (1962).
16. Skoog, D.A.; Holler, F.J.; Crouch, S.R. Gas Chromatography, in: Principles of Instrumental Analysis. 6th Edition (2007).
17. Millipore Sigma GC Column Selection Guide. https://www.sigmaaldrich.com/technical-documents/articles/analytical/gccolumn-selection-guide.html (accessed Nov 27, 2022).
18. Rahman, M. M.; Abd El-Aty, A. M.; Choi, J.; Shin, H.; Shin, S. C.; Shim, J. *Anal Separation Sci*. 2015, 823-834.
19. Restek Guide to GC Column Selection and Optimizing Separations. https://www.restek.com/globalassets/pdfs/literature/GNAR1724B-UNV.pdf
20. Supelco, GC Column Selection Guide. *LC GC North America*. 2012, 30, 523.
21. ThermoFisher, GC Detectors. https://www.thermofisher.com/ca/en/home/industrial/chromatography/gas-chromatography-gc/gc-detectors.html (Accessed Dec 27, 2022).
22. GenTech Scientific, Types of Detectors used in Gas Chromatography. https://gentechscientific.com/types-of-detectors-used-in-gas-chromatography/ (Accessed Dec 27, 2022)
23. James, A.T.; Martin, J.P. *Biochem J*. 1952, 50 (5), 679-690.
24. Bartle K.D.; Myers, P. *TrAC Trends in Anal Chem.* 2002, 21 (9-10), 547-557.
25. Wang Z, Fingas M. *J Chromatogr A*. 774 (1997), 51-78.
26. Liu, Z.; Phillips, J.B. *J of Chrom Sci.* 1991, 29 (6), 227-231.
27. Nam, S. L.; Tarazona Carrillo, K.; de la Mata, A. P.; de Bruin, O. M.; Doukhanine, E.; Harynuk, J. Metabolomics 2022, 18 (4).
28. Graves, B. M.; Johnson, T. J.; Nishida, R. T.; Dias, R. P.; Savareear, B.; Harynuk, J. J.; Kazemimanesh, M.; Olfert, J. S.; Boies, A. M. Scientific Reports 2020, 10 (1).
29. Muscalu, A.M.; Górecki, T. *Trends Anal Chem*. 106 (2018) 225-245.
30. Nizio, K.D.; McGinitie, M.M.; Harynuk, J.J. *J of Chromatogr*. A. 14 (2012) 12-23.

31. Maria, M.H.; Jørgenrud, B.M.; Berg, T. *J. Chrom. A.* 2022, 1684 (22), 463566.
32. Seeley, J. V.; Seeley, S. K. *Anal Chem* 2013, 85, 557-578.
33. Seeley, J.V.; Micyus, N.J.; McCurry, J.D.; Seeley, S.K. *American Laboratory*. 2006.
34. SepSolve Analytical, INSIGHT GC×GC. https://www.sepsolve.com/separation/separation.aspx (Accessed Oct 16, 2022).
35. Górecki, T. *The Analytical Scientist.* GC×GC: The Road Not Taken. 2021.
36. Johnson, T.A.; Armstrong, M.D.S.; de la Mata, A.P.; Harynuk, J.J. *J of Chromatography Open*. 2022, 2, 100070.
37. ScienceDirect, Flame Ionization Detector. 2018.
38. SepSolve Analytical, GC×GC with Parallel Detection. 2016, 006.
39. Vaye, O.; Ngumbu, R.; Xia, D. *Rev in Anal Chem.* 2022, 41 (1), 63-73.
40. Chun, S.; Muthu, M.; Gopal, J. *Chemosensors*. 2022, 10 (12), 530.
41. Lakshmipriya, M.; Kokilambigai, S.; Ilango, K. *R J of Pharmacognosy*. 2022, 10 (1), 57-66.
42. López-Ruiz, R.; Maldonado-Reina, A.J.; Marín-Sáez, J.; Romero-González, R.; Martínez-Vidal, J.L.; Frenicha, A.G. *Trends in Env Anal Chem.* 2023, 37, e00191.
43. Špánik, I.; Machyňáková, A. *J of Sep Sci*. 2017, 41 (1), 163-179.
44. Azo Materials, How GC×GC Increases Routine Laboratory Performance. https://www.azom.com/article.aspx?ArticleID=16323 (Accessed Dec 27, 2022).
45. Yang, X.; Wang, C.; Sao, H.; Zheng, Q. *Sci Total Env*. 694 (2019) 1-7.
46. Guo, Q.; Li, X.; Yu, J.; Zhang, H.; Zhang, Y; Yang, M.; Lu, N.; Zhang, D. *Anal Methods*. 7 (2015) 2458-2468.
47. Guo, Q.; Li, X.; Yu, J.; Zhang, H.; Zhang, Y.; Yang, M.; Lu, M.; Zhang, D. *Anal Methods*. 2015, 7, 2458-2468.
48. X. Yang, C. Wang, H. Sao, Q. Zheng. *Sci of the Total Env*. 2015, 694, 1-7.
49. Harris, D.C. Gas Chromatography, in: Quantitative Chemical Analysis. 6th Edition (2003).
50. Nichols, L. Extraction Theory, in: Organic Chemistry Lab Techniques. https://organiclabtechniques.weebly.com/ (Accessed Dec 20, 2022).
51. Rezaee, M.; Assadi, Y.; Hosseini, M.M.; Aghaee, E.; Ahmadi, F.; Berijani, F. *J Chrom A.* 2006, 1116, 1-9.
52. AGAT Environmental, Extraction and Analysis of Soil Samples by Liquid Extraction. 2014.
53. United States Environmental Protection Agency, Caltest Standard Operating Procedure EPA 3540C. 2009, 1-11.
54. ThermoFisher, GC Autosamplers. https://www.thermofisher.com/ca/en/home/industrial/chromatography/gas-chromatography-gc/gc-autosamplers.html (Accessed Dec 27, 2022).
55. Agilent, GC Sample Preparation & Introduction. https://www.agilent.com/en/product/gas-chromatography/gc-sample-preparation-introduction (Accessed Dec 27, 2022)
56. Labcompare, GC Autosampler / GC Headspace Autosampler https://www.labcompare.com/Laboratory-Analytical-Instruments/37-GC-Autosampler/ (Accessed Dec 27, 2022).
57. United States Environmental Protection Agency, Caltest Standard Operating Procedure EPA 325. 2015. 1-33.
58. Galuszka, A.; Migaszewski, Z.M.; Konieczka, P.; Namiesnik, J. *TrAC Trend Anal Chem.* 2012, 37, 61-72.
59. Woolfenden, E. Ch 10 – Thermal Desorption Gas Chromatography, in: *Handbooks in Separation Science*. 2021, 2, 267-323.
60. NEW – Janusz Book
61. Pawliszyn, J. Applications, in: Solid Phase Microextraction: Theory and Practice. 1st Edition, 1997.

62. D'Ovidio, C.; Bonelli, M.; Rosato, E.; Tartaglia, A.; Ulusoy, H.I.; Samanidou, V.; Furton, K.G.; Kabir, A.; Ali, I.; Savini, F.; Locatelli, M.; de Grazia, U. *Separations*. 2022, 9 (1), 18.
63. Olkowicz, M.; Rosales-Solano, H.; Ramadan, K.; Wang, A.; Cypel, M.; Pawliszyn. *Front Cell Dev Bio.* 2022, 10.
64. Millipore-Sigma, Solid Phase Micro-Extraction (SPME). https://www.sigmaaldrich.com/CA/en/products/analytical-chemistry/analytical-sample-preparation/spme-fibers-and-accessories. Accessed Jan 23, 2023.
65. United States Environmental Protection Agency, Index to EPA Test Methods. https://www.epa.gov/measurements-modeling/index-epa-test-methods (Accessed Dec 27, 2022).
66. Government of Canada, Guidelines for Canadian Drinking Water Quality (2022) https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html#t2, 2022 (accessed 30 July 2022).
67. Chen, Y.A.; Liu, P.W.G.; Whang, L.M.; Wu, Y.J.; Cheng, S.S. *J Biosci Bioeng.* 129 (2020) 603-612.
68. Van der Heul, R.M. Environmental Degradation of Petroleum Hydrocarbons. MS Thesis. Utrecht University (2009).
69. Kang, H.J.; Jung, Y.; Kwon, J.H. *Chemosphere*. 2019, 222, 656-664.
70. Wang, Z.; Fingas, M. *J Chromatogr A*. 1997, 774, 51-78.
71. Kutlucinar, K.G.; Handl, S.; Allabashi, R.; Causon, T.; Troyer, C.; Mayr, E.; Perfler, R.; Hann, S. *Env Sci Pollution Res.* 2022, 29, 64568-64581.
72. McCord, J.P.; Groff II, L.C.; Sobus, J.R. *Env Internat*. 2022, 158, 107011.
73. Hajeb, P.; Zhu, L.; Bossi, R.; Vorkamp, K. *Chemosphere.* 2022, 287 (3), 132306.
74. Sajid, M.; Płotka-Wasylkab, J. *Talanta*. 2022, 238 (2), 123046.
75. Wojnowski, W.; Tobiszewski, M.; Pena-Pereira, F.; Psillakis, E. *TrAC Trends Anal Chem.* 2022, 149, 116553.
76. Galuszka, A.; Migaszewski, Z.M.; Konieczka, P.; Namiesnik, J. *TrAC Trend Anal Chem.* 37 (2012) 61-72.
77. Wang, Z.; Fingas, M. *J. Chromatogr. A.* 774 (1997), 51-78.
78. Ma, R.; Ortiz, S. Preparation of CCME F2-4 and Total Extractable Hydrocarbons in Soil and Water. Standard Operating Procedure, AGAT Laboratories (2018).
79. Guo, Q.; Li, X.; Yu, J.; Zhang, H.; Zhang, Y.; Yang, M.; Lu, N.; Zhang, D. *Anal Methods.* 7 (2015) 2458-2468.
80. United States Environmental Protection Agency. 2010 U.S. EPA decontamination research and development conference Snyder report. 4303 (2010) 1-30.
81. Azimi, A.; Biakhtiari, A.R.; Tauler, R. *Env Pollution*. 243 (2018) 374-382.
82. Othmer, D.F.; White, R.E.; Trueger, E. *Ind Eng Chem.* 33, *10* (1941) 1240-1248.
83. Lee, S.; Kim, K.; Jeon, J.; Moon, H.B. *Ecotox And Env Safety.* 181 (2019) 11-17.
84. Yang, X.; Wang, C.; Sao, H.; Zheng, Q. *Sci of the Total Env.* 694 (2019) 1-7.
85. Gao, X.; Huang, P.; Huang, Q.; Rao, K.; Lu, Z.; Xu, Y.; Gabrielsen, G.W.; Hallanger, I.; Ma, M.; Wang, Z. *Env Pollution.* 253 (2019) 1-10.
86. Schemeth, D.; Nielsen, N.J.; Andersson, J.T.; Christensen, J.H. *Chemosphere*. 235 (2019) 175-184.
87. Avagyan, R.; Åberg, M.; Westerholm, R. *Chemosphere*. 163 (2016) 313-321.
88. Beens, J.; Blomberg, J.; Schoenmakers, P.J. *J High Resol Chromatogr.* 23 (2000) 182-188.
89. Yue, Z.; Shi, Q.; Ai, J.; Peng, S.; Miao, X.; Wang, Z. *IOP Conf Series: Earth and Env Sci.* 621 (2021) 012011.
90. Soon, Z.Y.; Loh, A.J.Y.; Joseph, C.G.; Sakari, M. *Life Sci J.* 18(4) (2021) 39-49.
91. Rezaee, M.; Assadi, Y.; Hosseini, M.M.; Aghaee, E.; Ahmadi, F.; Berijani, S. *J Chrom A.* 1116 (2006) 1-9.
92. Chou, T.Y.; Lin, S.L.; Fuh, M.R. *Talanta*. 80 (2009) 493-498.

93. Assadi, Y.; Farajzadeh, M.; Bidari, A. Dispersive Liquid-Liquid Microextraction, in: Comprehensive Sampling & Sample Preparation. 2 (2012) 181-207.
94. Assadi, Y.; Ahmadi, F.; Hosseini, M.M. *Chromatographia*. 71 (2010) 1137-1141.
95. Tobiszewski, M.; Bigus, P.; Namiesnik, J. *Anal Methods*, 6 (2014) 6678.
96. Muscalu, A.M.; Górecki, T. *Trends in Anal Chem.* 106 (2018) 225-245.
97. Nizio, K.D.; McGinitie, M.M.; Harynuk, J.J. *J of Chromatogr A.* 14 (2012) 12-23.
98. Harris, D.C. Quality assurance and calibration methods, in: Quantitative Chemical Analysis*, 9th Edition* (2015).
99. Glassdoor, Companies Reviews. https://www.glassdoor.ca/Reviews
100. Millipore-Sigma, Products. https://www.sigmaaldrich.com/CA/en/products/analytical-chemistry
101. Government of Alberta. Oil sands facts and statistics, https://www.alberta.ca/oil-sands-facts-and-statistics.aspx. Accessed Jan 20, 2023.
102. Statistics Canada. The Decline in Production and Investment in Canada's Oil and Gas Sector and its Impact on the Economy, https://www150.statcan.gc.ca/n1/pub/11-626-x/11-626-x2020007-eng.htm. Accessed Jan 20, 2023.
103. Hodson, P.V. *PNAS*. 2013, 110 (5), 1569-1570.
104. Leary, S. National Geographic. Environment: This is the world's most destructive oil operation—and it's growing. 2019. Accessed Jan 20, 2023.
105. Canadian Broadcasting Corporation. Alberta Pipelines: 5 Major Oil Spills in Recent History. https://www.cbc.ca/news/canada/alberta-pipelines-5-major-oil-spills-in-recent-history-1.3156604#:~:text=Almost%20half%20a%20million%20litres,near%20the%20community%20of%20Sundre.&text=The%20461%2C000%2Dlitre%20spill%20(about,criticism%20from%20Alberta's%20energy%20watchdog. Accessed Jan 20, 2023.
106. Natural Resources Canada. Alberta's Shale and Tight Resources. https://www.nrcan.gc.ca/energy/energy-sources-distribution/natural-gas/shale-tight-resources-canada/albertas-shale-and-tight-resources/17679. Accessed Jan 21, 2023.
107. Kinnaman, T.C. Bucknell Digital Commons, The Economic Impact of Shale Gas Extraction: A Review of Existing Studies. 2010.
108. Environment and Natural Resources Canada. List of Toxic Substances Managed Under Canadian Environmental Protection Act. https://www.canada.ca/en/environment-climate-change/services/management-toxic-substances/list-canadian-environmental-protection-act.html. Accessed Jan 21, 2023.
109. Canadian Association for Laboratory Accreditation. CALA Directory of Laboratories. https://directory.cala.ca/directory-search. Accessed Jul 30, 2022.
110. Saari, E.; Perämäki, P.; Jalonen, J. *Microchimica Acta.* 2007, 158, 261-268.
111. Google Trends. Soxhlet. https://trends.google.com/trends/explore?date=all&geo=CA&q=Soxhlet. Accessed Jan 22, 2023.
112. Li, X.; Du, Y.; Wu, G.; Li, Z.; Li, H.; Sui, H. *Chemosphere*. 2012, 88 (2), 245-249.
113. United States Environmental Protection Agency, Method 3540C. https://www.epa.gov/sites/default/files/2015-12/documents/3540c.pdf. Accessed Jan 22, 2023.
114. Piotrowski, P. K.; Tasker, T. L.; Burgos, W. D.; Dorman, F. L. *J. Chromatogr. A.* 2018, 1579, 99-105.
115. Nam, S.L.; de la Mata, A.P.; Harynuk, J.J. *Multidimen Sep Metabol.* 2021, 8 (6), 84.
116. GitHub. Ryland-chem, importPeakTables. https://github.com/ryland-chem/importPeakTables. Last edited Jan 18, 2023.
117. Galuszka, A.; Migaszewski, Z.M.; Konieczka, P.; Namiesnik, J. *TRAC Trend Anal Chem.* 2012, 37, 61-72.
118. Sigma-Aldrich. Solvents. https://www.sigmaaldrich.com/CA/en/products/analytical-chemistry/analytical-chromatography/solvents. Accessed Jan 20, 2023.

119. Pan, Y.; Zeng, X.; Xu, H.; Sun, Y.; Wang, D.; Wu, J. *J of Hydrology.* 2020, 584, 124690.
120. Rohan, R. Analyzing the Potential Impact of DNAPL in Groundwater Contamination. 2022. https://www.researchgate.net/profile/Ritik-Rohan/publication.
121. Schaefer, C.E.; Lavorgna, G.M.; Haluska, A.A.; Annable, M.D. *Groundwater Monitoring and Remediation*. 2018, 38 (3), 65-74.
122. Chapman, S.W.; Parker, B.L. *Water Res Research.* 2005, 41 (12).
123. Guadaño, J.; Gómez, J.; Fernández, J.; Lorenzo, D.; Dominguez, C.M.; Cotillas, S.; Garcia-Cervilla, R.; Santos, A. *Sustainability.* 2022, 14 (24), 16576.
124. https://www.sourcewaterinfo.on.ca/images/uploaded/uploadedDownloads/DNAPL-Fact-Sheet-CO-ABMV-April-2015-web.pdf. Accessed Dec. 11, 2022.
125. Engelmann, C.; Händel, F.; Binder, M.; Kumar Yadav, P.; Dietrich, P.; Liedl, R.; Walther, M. *J of Hazardous Materials.* 2019, 375, 233-240.
126. Harris, D.C.; Lucy, C.A. Gas Chromatography, in: Quantitative Analysis, 10th Edition (2020).
127. Tong, Y.; Karasek, F.W. *Anal Chem*. 1984, 56, 2124-2128.
128. Brown, D.G.; Gupta, L.; Moo-Young, H.K.; Coleman, A.J. Env Toxicol and Chem. 2005, 24 (8), 1886-1892.
129. Pirbazari, M.; Borow, H.S.; Craig, S.; Ravindran, V.; McGuire M.J. *Wat. Sci. & Tech.* 25, *2*. (1992) 81-88.
130. Liato, V.; Aider M. *Chemosphere*. 181 (2017) 9-18.
131. Watson, S.B.; Brownlee, B.; Satchwell, T.; Hergesheimer, E.E. *Wat. Res.* 34, *10.* (2000) 2818-2828.
132. Bruce, D.; Westerhoff, P.; Brawley-Chesworth, A. *J. of Water Supply: Research and Technology-Aqua.* 2002, 51 (4), 183-198.
133. Lindholm-Lehto, P.C.; Vielma, J. *Aquaculture Research*. 2019, 50 (1), 9-28.
134. Mustapha, S.; Tijani, J.O.; Ndamitso, M.M.; Abdulkareem, A.S.; Shuaib, D.T.; Mohammed, A.K. *Environ Monit and Assess.* 2021, 193, 204.
135. Gorzynski Smith, J. Stereochemistry, in: Organic Chemistry, 3rd Edition (2011).
136. (±)-geosmin. Safety Data Sheet, Santa Cruz Biotechnology, Inc. 2016, 1.1.
137. 1,2-dimethylenecyclohexane. Safety Data Sheet, UN GHS. 2019, 1.0 (9).
138. 1,2-dimethylcyclohexane, mixture of cis and trans. Safety Data Sheet, ThermoFisher Scientific. 2009, 5.
139. Nichols, L. Extraction Theory, in: Organic Chemistry Lab Techniques. https://organiclabtechniques.weebly.com/ (Accessed Dec 20, 2022).
140. Hoque, M.S.; Saha, A.; Chung, H.J.; Dolez, P.I. *J Appl Polym Sci*. 2022, 139 (30).
141. Ashbrook, A.W. *Talanta*. 1975, 22 (4-5), 327-343.
142. Poole, C.F. Milestones in the Development of Liquid-Phase Extraction Techniques, in: Liquid-Phase Extraction. 1st Edition, 2020.
143. Gao, X.; Huang, P.; Huang, Q.; Rao, K.; Lu, Z. Xu, Y.; Gabrielsen, G.W.; Hallanger, I.; Ma, M.; Wang, Z. *Env Pollution*. 253 (2019) 1-10.
144. Schemeth, D.; Nielsen, N.J.; Andersson, J.T.; Christensen, J.H. *Chemosphere*. 235 (2019) 175-184.
145. Avagyan, R.; Åberg, M.; Westerholm, R. *Chemosphere*. 163 (2016) 313-321.
146. Beens, J.; Blomberg, J.; Schoenmakers, P.J. *J High Resol Chromatogr.* 23 (2000) 182-188.
147. Mazur, D.M., Latkin, T.B., Kosyakov, D.S., Kozhevnikov, A.Yu., Ul'yanovskii, N.V., Kirilov, A.G., Lebedev, A.T. (2020). *Env Pollution*, 265(B), 114885.
148. Chan, J.X.; Hassan, A.; Wong, J.F.; Majeed, K. *Encyclopedia of Materials: Plastics and Polymers.* 2022, 4, 237-248.

149. Farn, R.J. Non-ionic surfactants, in Chemistry and Technology of Surfactants. 2006.
150. Yang, Y.; Yu, J.; Xu, H.; Sun, B. Applications: Current Status and Future Prospects, in: Porous Lightweight Composites Reinforced with Fibrous Structures. 2017.
151. White, P.F.; Eng, M.R. Intravenous Anesthetics, in: Clinical Anesthesia. 6th Ed. 2009.
152. Bruker, P.; Khan, K. Practical Sports Medicine, in: Clinical Sports Medicine. 3rd Ed. 2007.
153. Gorzinski Smith, J. Reactions of Carboxylic Acids; Malonic Ester Synthesis; Oxidation and Reduction of Substituted Benzenes, in: Organic Chemistry. 3rd Ed. 2011.
154. Claassen, V. Intravenous Drug Administration, in Techniques in the Behavioral and Neural Sciences. 1994, 12, 5-22.
155. Baker, D.R.; Kasprzyk-Hordern, B. *J of Chrom A.* 2011, 1218 (12), 1620-1631.
156. Pérez-Baeza, M.; Escuder-Gilabert, L.; Martín-Biosca, Y.; Sagrado, S.; José, Medina-Hernández. *J of Chrom A.* 2020, 1625, 461334. M.J.
157. Yao, B.; Lian, L.; Pang, W.; Yin, D; Chan, S.A.; Song, W. *Chemosphere.* 2016, 160, 208-215.
158. Casado, J.; Brigden, K.; Santillo, D.; Johnston, P. *Sci of the Total Env.* 2019, 670, 1204-1225.
159. Ferreira, J.M.; Jorge, S. Félix, L.; Morello, G.M.; Olsson, I.A.S.; Valentin, A.M. *Biology*. 2022, 11, 1433.
160. Hasei, M.; Hirata, T.; Nishihara, H.; Tanigami, H.; Takashina, M.; Mori, T. *The Japanese J of Asenthesiology.* 2003, 52 (4), 394-398.
161. King, C.; McCue, A.; *Nursing Ethics*. 2017, 24 (4), 452-461.
162. Safe Disposal of Prescription Drugs, Health Canada, https://www.canada.ca/en/health-canada/services/safe-disposal-prescription-drugs.html. Accessed 19 Dec, 2022.
163. Sewage Disposal, Open Data, Open Government Portal, https://search.open.canada.ca/en/od/?od-search-portal=Open+Data&search_text=sewage. Accessed 19 Dec, 2022.
164. Kumblathan, T.; Liu, Y.; Uppal, G.K.; Hrudey, S.E.; Li, X.F. *ACS Environ Au*. 2021, 1 (1), 18-31.
165. Daughton, C.G. *Sci of the Total Environ.* 2018, 619-620, 748-764.
166. Pawliszyn, J. Applications, in: Solid Phase Microextraction: Theory and Practice. 1st Edition, 1997.
167. Majedi, S.M.; Lee, H.K. Microextraction and Solventless Techniques, in: The application of Green Solvents in Separation Processes, *Elsevier*. 2017, 415-450.
168. Lorenzo-Parodi, N.; Kaziur, W.; Stojanović, N.; Jochmann, M.A.; Schmidt, T.C. *TrAC Trends Anal Chem.* 2019, 113, 321-331.
169. Arthur, C.L.; Pawliszyn, J. *Anal Chem*. 1990, 62 (19), 2145-2148.
170. Shellie, R.A. Methods, in Encyclopedia of Forensic Sciences, 2nd Edition. 2013, 579-585.
171. Liang, S.Y.; Shi, F.; Zhao, Y.G.; Wang, H.W. *Molecules.* 2022, 27 (12), 5509.
172. Lord, H.L.; Zhang, X.; Musteata, F.M.; Vuckovic, D.; Pawliszyn, J. *Nat Protoc.* 2011, 6 (6), 896-924.
173. Watson, S.B.; Brownlee, B.; Satchwill, T.; Hargesheimer, E.E. *Wat Res*. 2000, 34, 2818-2828.
174. Mosekiemang, T.T.; Stander, M.A.; de Villiers, A. *Chemosphere.* 2019, 220, 983-992.
175. Kovats, E. *Helvetica*. 1958, 41 (7), 1915-1932.
176. Urbaneja-Bernat, P.; Cloonan, K.; Zhang, A.; Salazar-Mendoza, P.; Rodriguez-Saona, C. Journal of Pest Science 2021, 94 (4), 1249–1263.
177. Porto-Figueira, P.; Figueira, J. A.; Berenguer, P.; Câmara, J. S. Food Chemistry 2018, 245, 141–149.
178. Pico, J.; Gerbrandt, E. M.; Castellarin, S. D. Food Chemistry 2022, 368, 130812.
179. Bederska-Łojewska, D.; Pieszka, M.; Marzec, A.; Rudzińska, M.; Grygier, A.; Siger, A.; Cieślik-Boczula, K.; Orczewska-Dudek, S.; Migdał, W. Molecules 2021, 26 (24), 7446.
180. Mildner-Szkudlarz, S.; Różańska, M.; Gaca, A.; Jeleń, H. H. LWT 2021, 148, 111718.
181. Rahman, F. U.; Nawaz, M. A.; Liu, R.; Sun, L.; Jiang, J.; Fan, X.; Liu, C.; Zhang, Y. Food Science and Technology 2022, 42.
182. Pagès-Hélary, S.; Dujourdy, L.; Cayot, N. Molecules 2021, 26 (20), 6254.

183. Rice, S.; Maurer, D.; Fennell, A.; Dharmadhikari, M.; Koziel, J. Molecules 2019, 24 (3), 536.
184. Sun, L.; Zhu, B.; Zhang, X.; Wang, H.; Yan, A.; Zhang, G.; Wang, X.; Xu, H. Scientific Data 2020, 7 (1).
185. Dymerski, T.; Namieśnik, J.; Leontowicz, H.; Leontowicz, M.; Vearasilp, K.; Martinez-Ayala, A. L.; González-Aguilar, G. A.; Robles-Sánchez, M.; Gorinstein, S. Food Research International 2016, 83, 74–86.
186. Dias, R.P.; Johnson, T.A.; Ferrão, L.F.V.; Munoz, P.R.; de la Mata, A.P.; Harynuk, J.J. *J of Chrom Open.* 2022, 100075.
187. Government of Canada, Guidelines for Canadian Drinking Water Quality. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html. Accessed Jan 26, 2023.

APPENDIX A: Sampling Locations and GC×GC-FID Chromatograms for Real Sample Analysis Profiled in CHAPTER 2

A

Samples 1-13 were collected between 10:00 and 17:00 on August 4, 2021. Conditions varied between sunny and overcast.

1. 200 m upstream of the water treatment plant outflow. Very clear in appearance.
2. 100 m upstream of the water treatment plant outflow. Very clear in appearance.
3. Immediately upstream of the treatment plant outflow where visible mixing and foaming was occurring between the outflow and river.
4. Immediately upstream of the treatment plant outflow, along the beach where no visible mixing was occurring between the outflow and river.
5. Downstream of the treatment plant outflow where visible mixing was occurring between the outflow and river. Frothing and foaming, strong odor.
6. 20 m downstream of the water treatment plant outflow, along the beach where no visible mixing was occurring between the outflow and river. Appeared clear and had no notable odor.
7. 200 m downstream of the water treatment plant outflow. Water was visually very clear.
8. Freshwater pond in a park nearby the water treatment plant. Green in colour and had a "dead vegetation" odor.
9. Freshwater pond in a park nearby the water treatment plant (immediate neighbor to the pond in 8). Green/orange in colour and had a "dead vegetation" odor.
10. Storm drain runoff from East of Gold Bar residential area. Murky brown in colour with a slight odor.
11. Creek immediately downstream of storm drain runoff. Clean in appearance.
12. Creek 100 m downstream of storm drain runoff. Clean in appearance.
13. Creek 200 m downstream of storm drain runoff. Clean in appearance.

Samples 14-17 were collected between 13:00 and 14:00 on August 20, 2021.

14. Sink tap from laboratory on 3rd floor west wing of Gunning-Lemieux Chemistry Centre. Appeared clean.
15. Drinking fountain on 3rd floor west wing of Gunning-Lemieux Chemistry Centre. Clean in appearance, but had a "plastic-like" odor.
16. Safety shower from laboratory on 3rd floor west wing of Gunning-Lemieux Chemistry Centre. Orange in colour with no notable odor.
17. "Clean" sample as analyzed by routine analysis company (undisclosed location). Some small chunks of organic matter floating in solution. Light green in appearance. Strong odor.

Samples 18-26 were collected between October 1 and October 2, 2021. Conditions were consistently overcast.

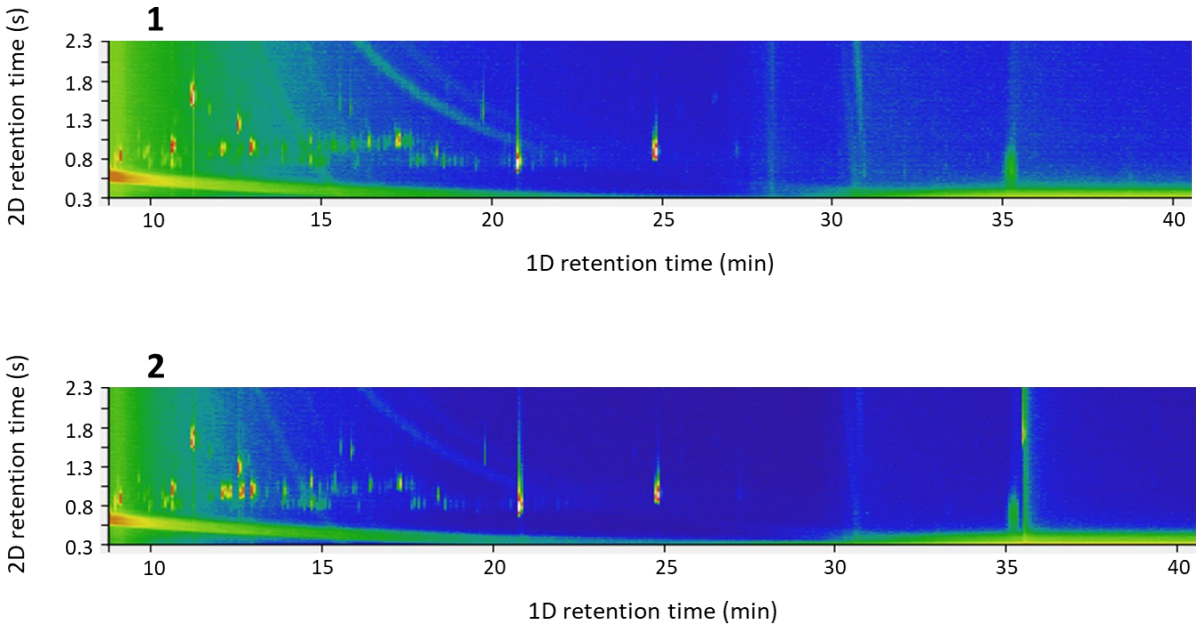
18. Downstream (~750 m) of a small oil leak from an overturned transport truck in southwest Edmonton. Inner bank of the stream. Clear in appearance, but a slight "oily" odor.

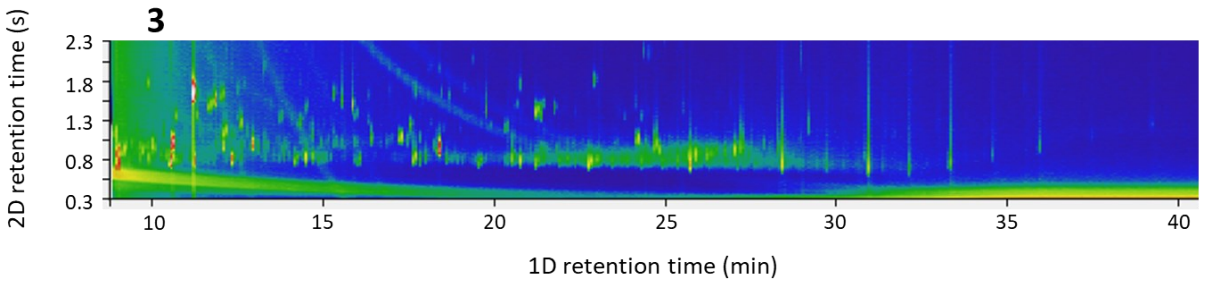
19. Downstream (~200 m) of a small oil leak from an overturned transport truck in southwest Edmonton. Outer bank of the stream. Very small droplets floating on surface of sample. Strong "oily" odor.
20. Sink tap of an untreated water source (bathroom) in the town of Ardrossan, East of Edmonton. Clean in appearance.
21. Sink tap of a treated/filtered water source (kitchen) in the town of Ardrossan, East of Edmonton. Clean in appearance.
22. Sink tap of a treated/filtered water source (kitchen) in the community of Hastings Lake, Southeast of Edmonton. Clean in appearance.
23. Sink tap of an untreated water source (industrial workshop) in the community of Hastings Lake, Southeast of Edmonton. Clean in appearance.
24. Rain barrel collected from a shingle roof in the community of Hastings Lake. Slightly green in appearance. Vegetation scent.
25. Ground water from a water pump in the community of Hastings Lake. Clean in appearance. Had a slight earthy odor.
26. Lake water from the Southeast side of Hastings Lake. Some organic matter suspended in solution. No notable odor.

Sample 27 was collected at approximately 13:00 on October 3, 2021.

27. Sink tap from the South-central side of the town of Camrose, Southeast of Edmonton. Clear in appearance.

B

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2D retention time (s)
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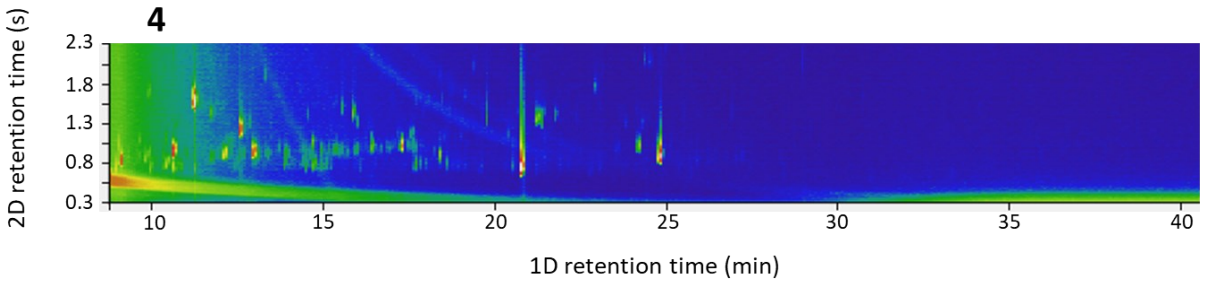
40
1D retention time (min)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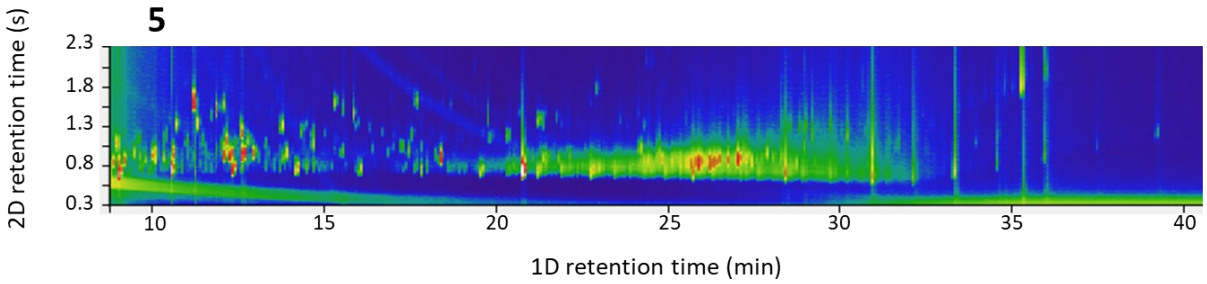
1D retention time (min)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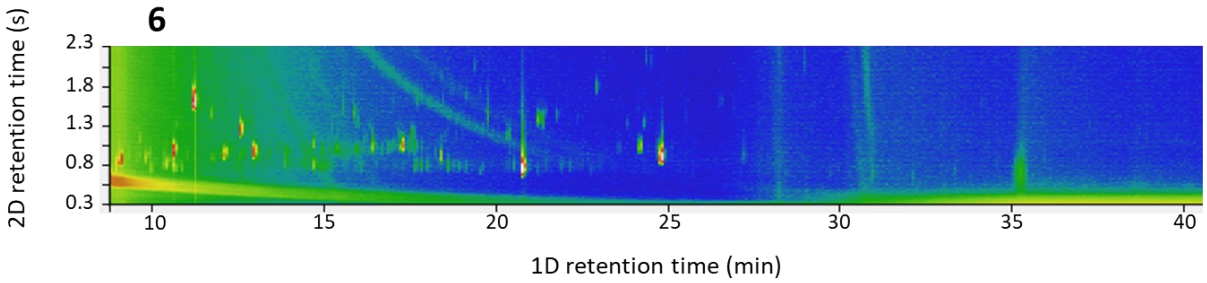
1D retention time (min)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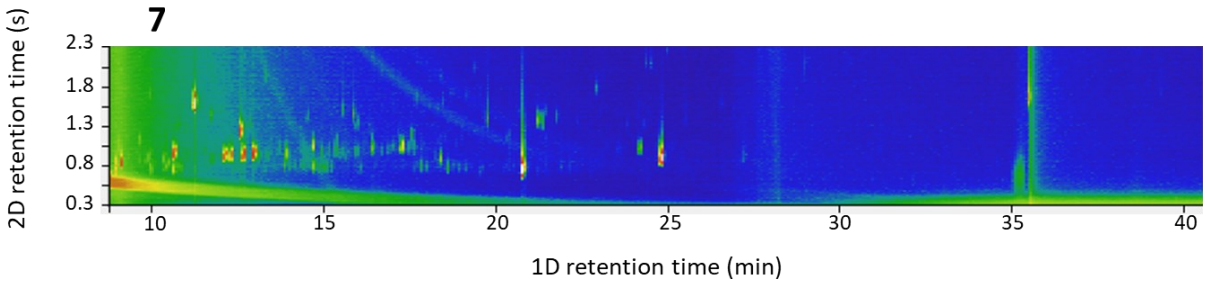
1D retention time (min)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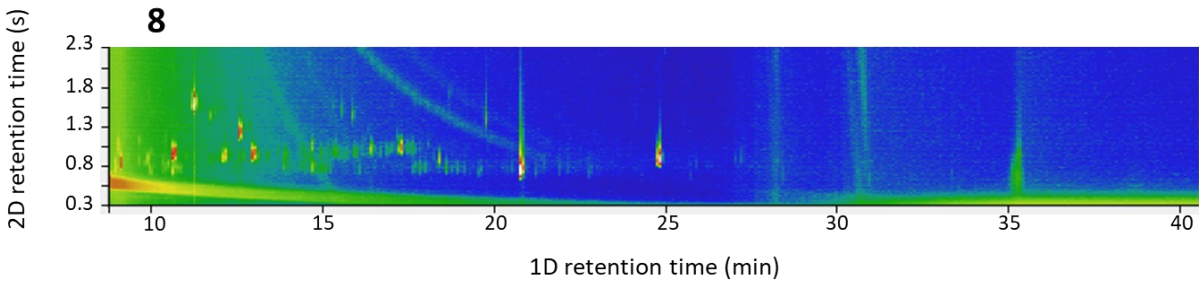
1D retention time (min)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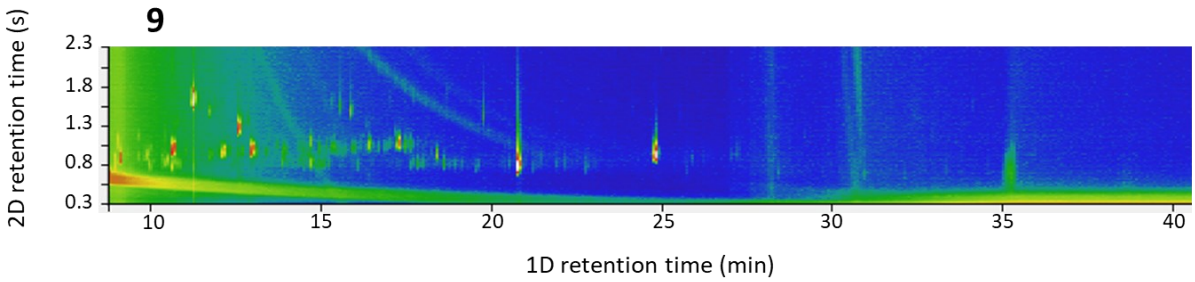
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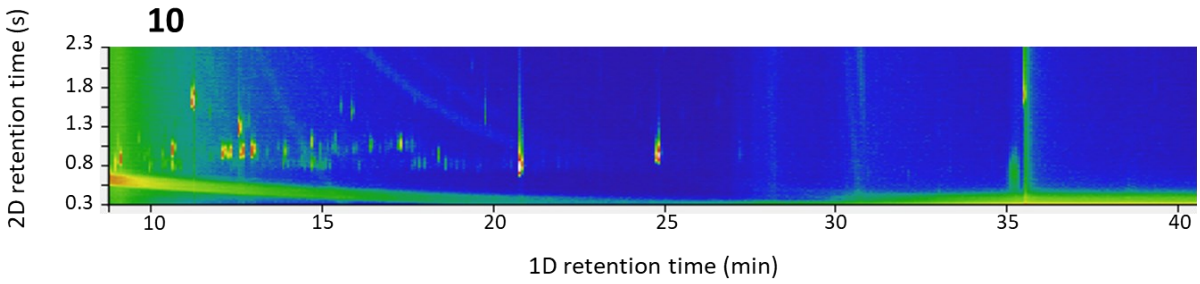
1D retention time (min)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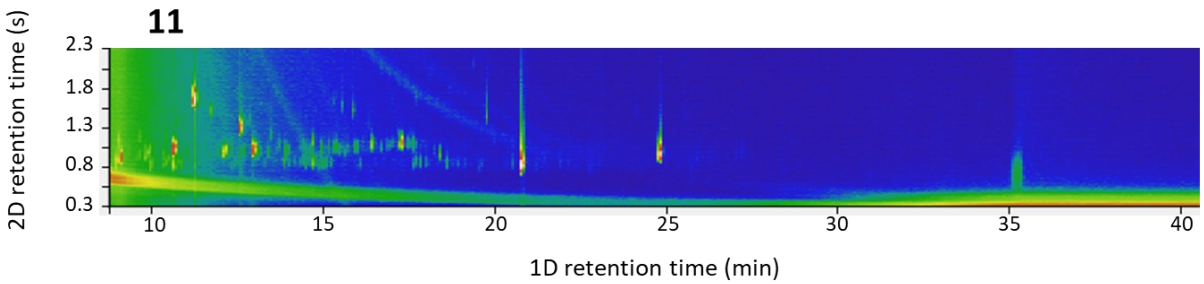
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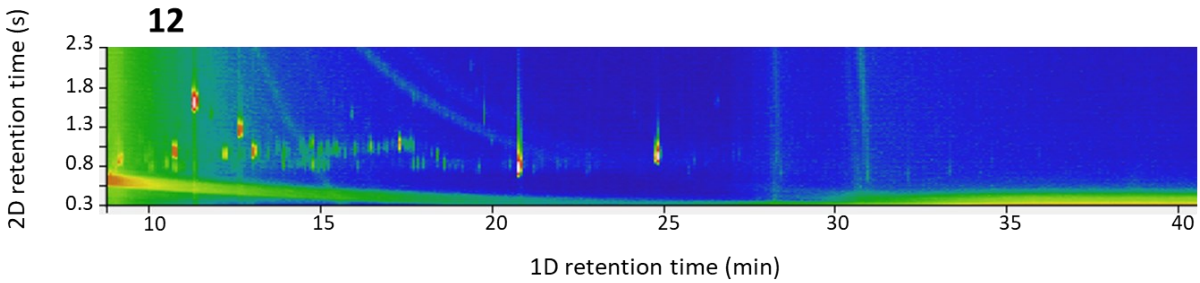
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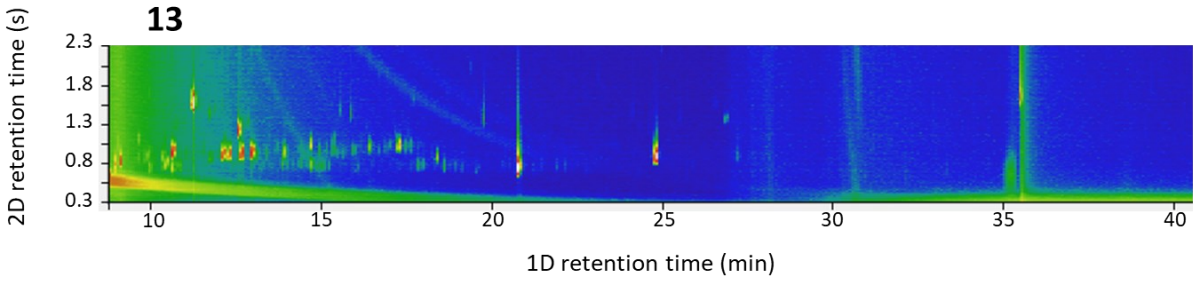
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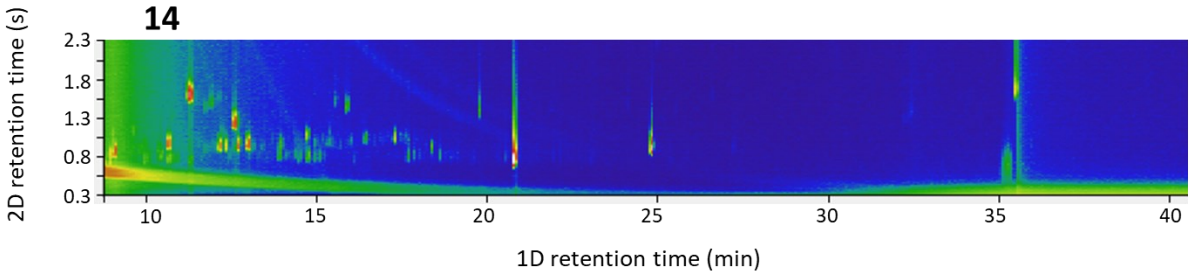
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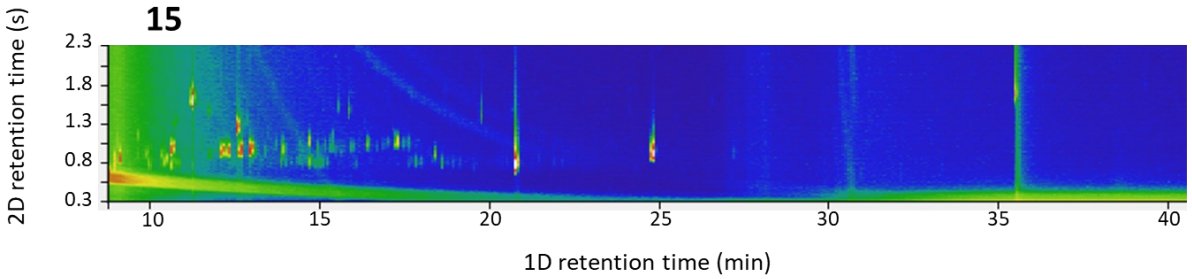
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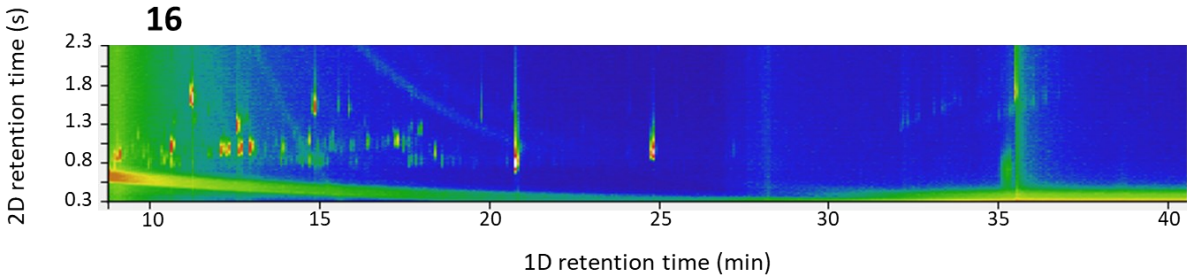
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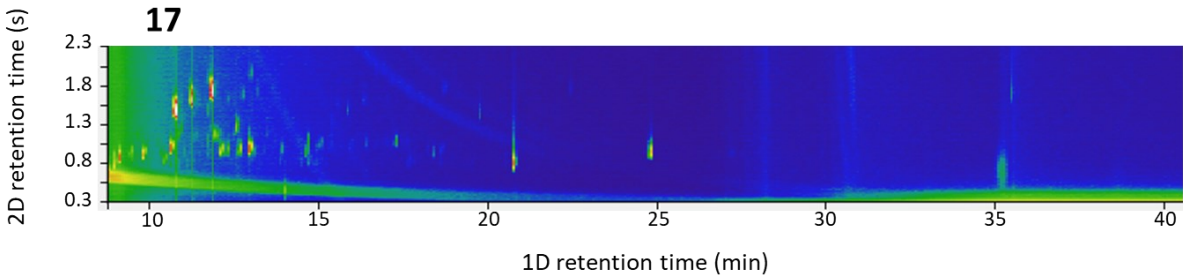
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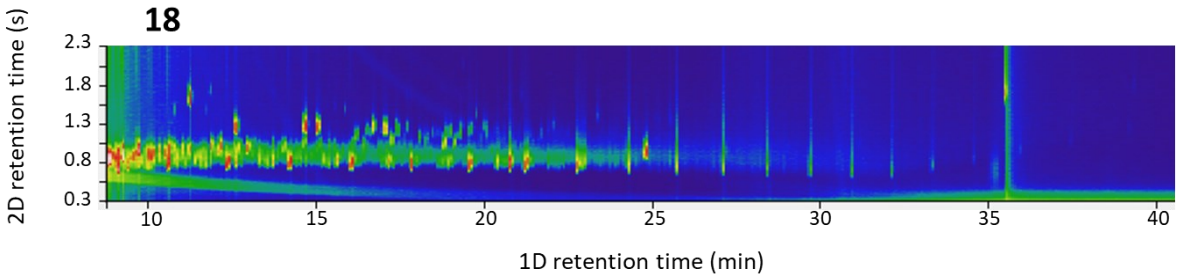
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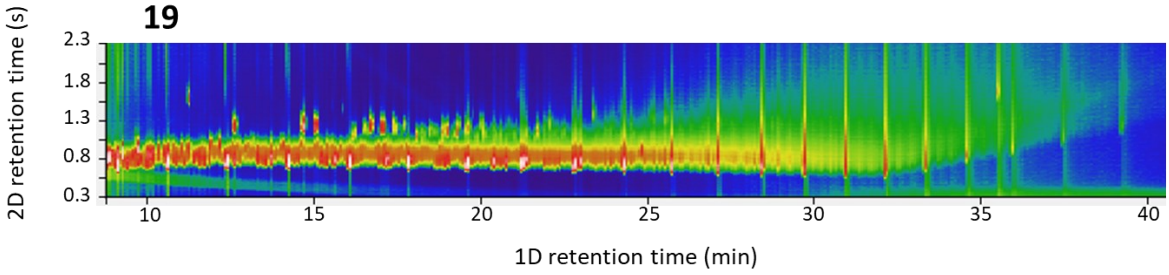
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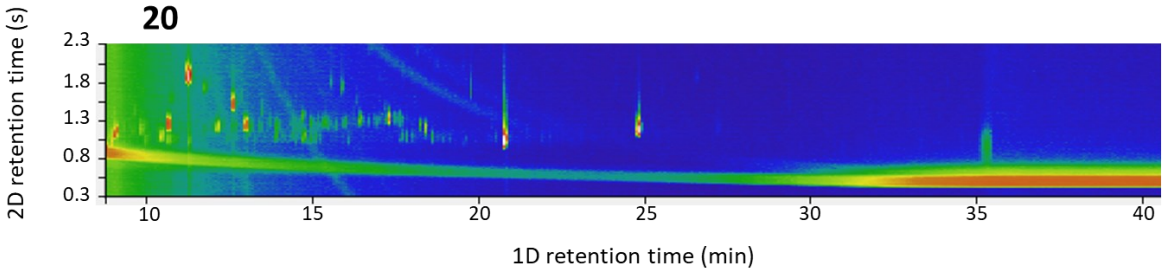
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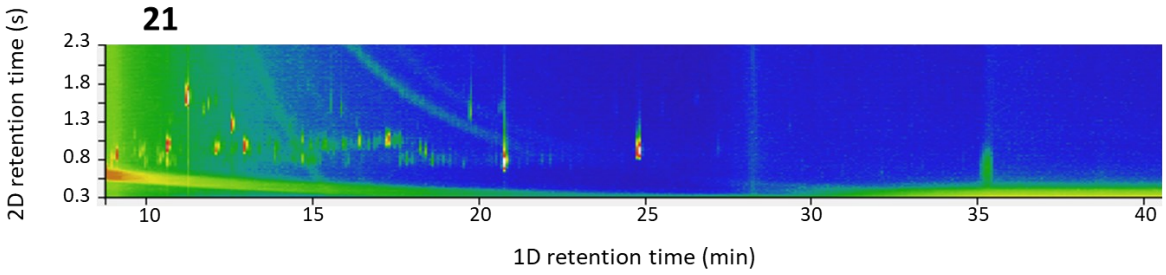
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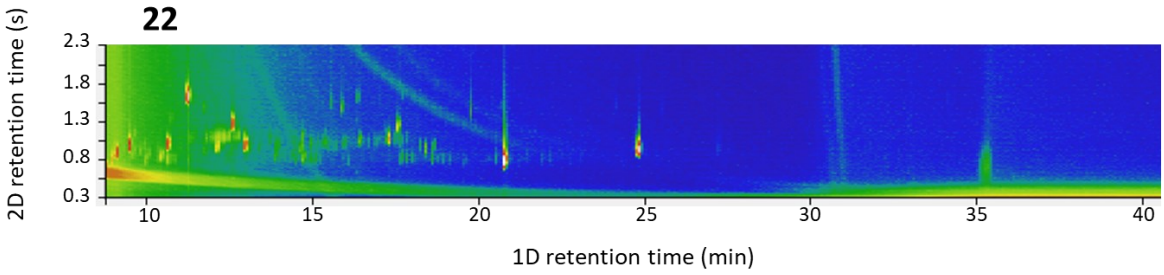
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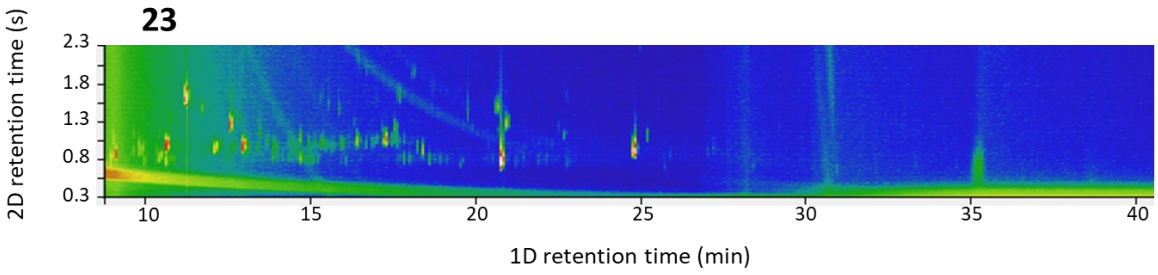
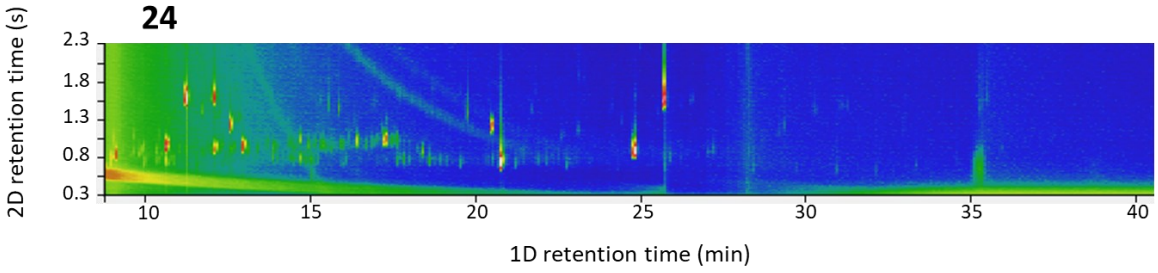
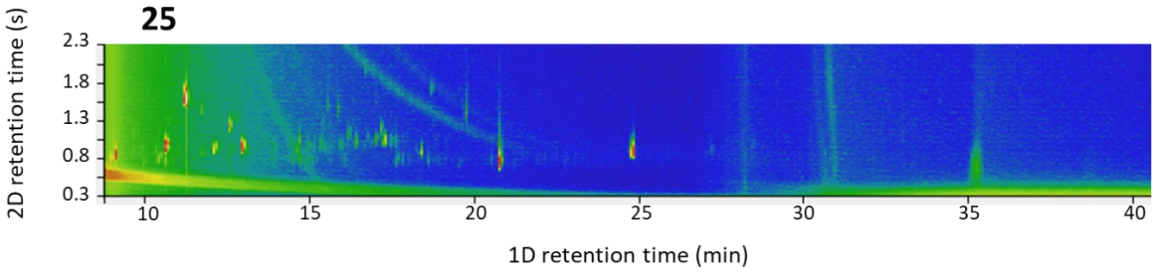
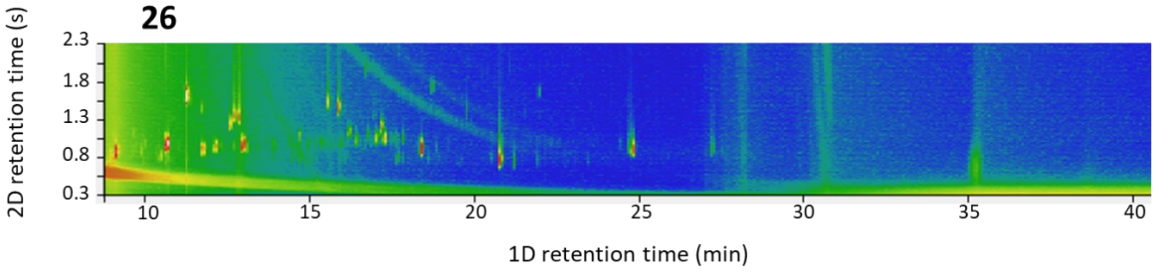
2D retention time (s)
2.3
1.8
1.3
0.8
0.3
10
15
20
25
30
35
40
1D retention time (min)


23
2D retention time (s)
2.3
1.8
1.3
0.8
0.3
10
15
20
25
30
35
40
1D retention time (min)


24
2D retention time (s)
2.3
1.8
1.3
0.8
0.3
10
15
20
25
30
35
40
1D retention time (min)


25
2D retention time (s)
2.3
1.8
1.3
0.8
0.3
10
15
20
25
30
35
40
1D retention time (min)


26
2D retention time (s)
2.3
1.8
1.3
0.8
0.3
10
15
20
25
30
35
40
1D retention time (min)

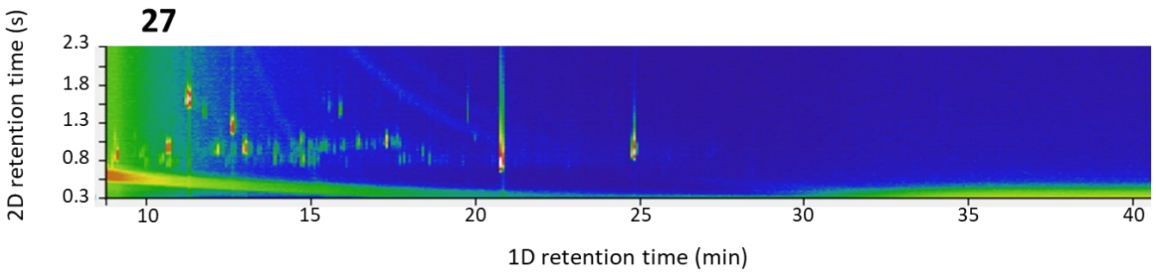


Figure A-1. A. A comprehensive list of the sampling locations and the conditions present. B. GC×GC-FID chromatograms for each of the 27 real samples analyzed.

APPENDIX B: Limit of Detection Information for Calibration Curves used in CHAPTER 4

Calibration Curve Compound	LOD y	LOD µg/mL	LOQ y	LOQ µg/mL	Line of Best Fit Equation
Chloroform	1.45E+06	1.14	3.75E+06	3.45	y = 1E+06x + 306076
1,2-dichloroethane	1.44E+06	0.59	2.42E+06	0.83	y = 4E+06x - 914883
3-methyl-2-pentanone	1.15E+06	0.26	1.50E+06	0.33	y = 5E+06x - 129518
Benzene	1.23E+06	0.10	3.08E+06	0.29	y = 1E+07x + 206689
1,2-dichloropropane	5.01E+06	0.48	5.14E+06	0.49	y = 6E+06x + 471502
Toluene	2.66E+06	0.21	8.50E+06	0.80	y = 1E+07x + 518726
Tetrachloroethylene	2.18E+06	0.29	7.20E+06	1.55	y = 4E+06x + 1E+06
Ethyl benzene	5.15E+05	0.12	1.54E+06	0.22	y = 1E+07x - 709129
o-xylene	4.67E+05	0.10	1.47E+06	0.20	y = 1E+07x - 528203

Table B-1. Compounds and their equations used for the calculation of relative concentrations of compounds in DNAPL mixtures. LOD calculated values were obtained using the formula $y_{dl} = 3 \times s + y_{bl}$ as described in Chapter 4.

APPENDIX C: Tentative Mass Spectral Identification Information of Fabric Samples Showcased in CHAPTER 6

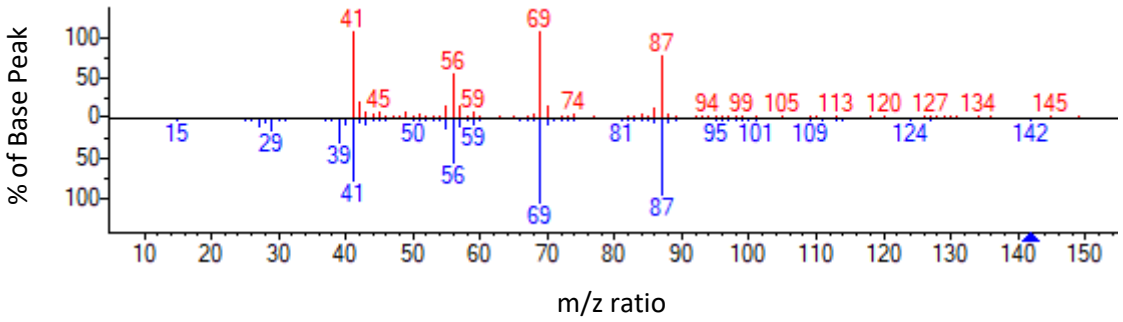


Figure C-1. The mass spectrum obtained for the peak of interest (top/red) and the mass spectral library match (bottom/blue) for n-butylmethacrylate. The match factor was ____

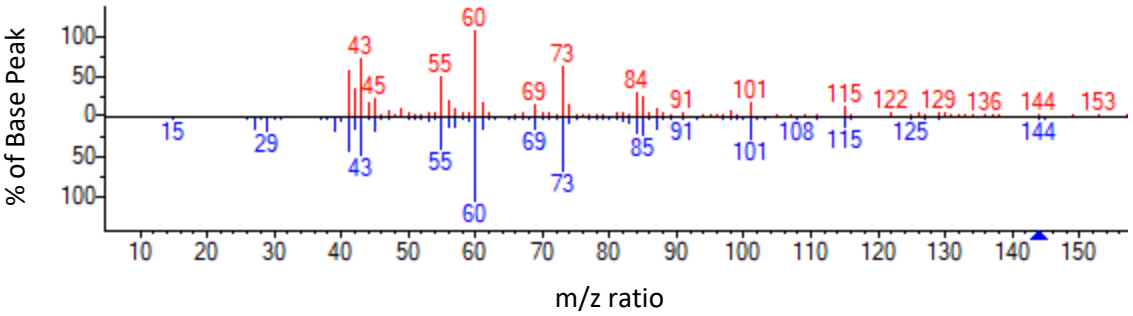


Figure C-2. The mass spectrum obtained for the peak of interest (top/red) and the mass spectral library match (bottom/blue) for octanoic acid. The match factor was ____

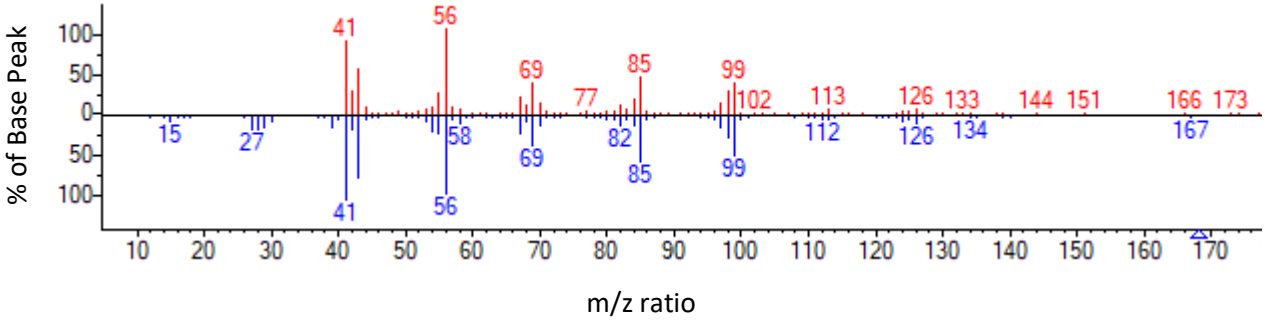


Figure C-3. The mass spectrum obtained for the peak of interest (top/red) and the mass spectral library match (bottom/red) for 1,6-diisocyanatohexane. The match factor was ____